Université de Toulouse

THÈSE

En vue de l'obtention du

DOCTORAT DE L'UNIVERSITÉ DE TOULOUSE

Délivré par :

Université Toulouse 3 Paul Sabatier (UT3 Paul Sabatier)

Présentée et soutenue par :
Paul Monsarrat

Le lundi 25 janvier 2016

Titre :

Cellules souches, médecine régénérative et régénération parodontale

---

ED BSB : Physiopathologie

Unité de recherche :

UMR StromaLab, CNRS 5273, UT3 Paul Sabatier, INSERM U1031, EFS Pyrénées-Méditerranée, FR

Directeur(s) de Thèse :

Dr Valérie Planat-Bénard et Pr Philippe Kémoun

Rapporteurs :

Pr Jean-Christophe Farges et Pr Hervé Petite

Autre(s) membre(s) du jury :

Pr Louis Casteilla

Remerciements

Professeur Jean-Christophe Farges et Professeur Hervé Petite,

Nous vous remercions chaleureusement d'avoir accepté d'être rapporteurs de ce travail et de siéger à notre jury de thèse. Vos conseils ont permis, je l'espère, d'améliorer significativement ce manuscrit. Veuillez trouver ici l'expression de notre sincère reconnaissance.

Professeur Louis Casteilla,

Nous vous remercions infiniment pour la chance que nous avons eu de travailler au sein de votre laboratoire. Sachez que votre aide scientifique inestimable, votre écoute attentive et votre disponibilité, nous ont touché au plus haut degré. J'espère que transparaissent dans ce travail, notre profonde gratitude et notre plus grand respect, en cet exemple de modèle d'intelligence scientifique et de mentor que vous incarnez. Nous espérons avoir la chance de pouvoir continuer auprès de votre équipe.

Docteur Valérie Planat-Bénard,

Nous vous remercions pour l'honneur que vous nous avez fait en acceptant de co-diriger cette thèse. Merci pour votre disponibilité, votre gentillesse et votre bonne humeur en toutes circonstances. Votre rigueur scientifique, les conseils que vous nous avez transmis, nous ont profondément inspiré, et jalonnerons ainsi notre parcours. Nous espérons pouvoir bénéficier encore de vos lumières, et continuer à travailler avec vous.

Professeur Philippe Kémoun,

Que ce soit votre rigueur dans la recherche, votre passion, vos enseignements et vos qualités humaines en général, vous êtes un exemple pour nous. Merci d'avoir été un œil bienveillant durant ces années, de nous avoir donné notre chance et fourni un cadre, d'avoir su nous transmettre également beaucoup de votre amour de la recherche. Nous espérons que ce travail témoigne de notre profonde reconnaissance et de notre haute considération.

A tout le laboratoire STROMALab,

Pour son accueil chaleureux, ses conseils, son aide inestimable et tous les bons moments partagés.

A mes collègues et mentors des 5x.03,

Pour leur soutien indéfectible, leurs conseils inestimables, la joie de travailler en équipe.

A ma Famille,
Merci d'avoir toujours cru en moi,
De m'avoir accompagné et soutenu tout au long de mes années d'étude.

A Lucie,
Pour ton amour et ta patience.

Table des matières

Partie II
Parodontite et régénération par l'utilisation des CSMs

Conclusion

Liste des abréviations

1-MT: 1-methyl-tryptophan
Alpha-MEM: alpha Minimum Essential Medium
ASC: Adipose Derived Mesenchymal Stromal Cell
ATP: Adenosine Tri-Phosphate
BM-MSC: Bone Marrow Mesenchymal Stromal Cell
BMMNC: Bone Marrow MonoNuclear Cell
BMP: Bone Morphogenetic Protein
BPI: Bactericidal/Permeability-Increasing protein
BSA : Bovine Serum Albumin
CFSE : Carboxyfluorescein Succinimidyl Ester
CSM : Cellule Stromale Mésenchymateuse
CTD : ClinicalTrials.gov
CTGF : Connective Tissue Growth Factor
CXCL : Chemokine (C-X-C motif) ligand
DiOC6(3): 3,3'-Dihexyloxacarbocyanine Iodide
DO: Densité Optique
DTD: Document Type Definition
ESC: Embryonic Stem Cell
EPC : Endothelial Progenitor Cell
FGF: Fibroblast Growth Factor
FSC: Forward Scatter
GFP: Green Fluorescent Protein
GMP: Good Manufacturing Practices
GvHD : Graft-vs-host Disease
H2DCFDA : 2',7'-dichlorodihydrofluorescein diacetate
Hba1c: Hémoglobine Glyquée
hESCs-RPE : Retinal Pigment Epithelium cells from Human Embryonic Stem cells
HLA: Human Leukocyte Antigen
HGF : Hepatocyte Growth Factor
HSC : Hematopoïétic Stem Cell
HT : Hough Transform
ICMJE : International Committee of Medical Journal Editors
IDO : InDoléamine Oxydase
IL : Interleukin
IMC : Indice de Masse Corporelle
iNOS: inducible Nitric Oxide Synthase
iPS : induced pluripotent stem cell
IP: Iodure de Propidium
KDD : Knowledge Discovery in Databases
LL-37 : human cationic antimicrobial protein (hCAP, CAMP)
LNAME : L -arginine methyl ester hydrochloride
MCP-1 : Monocyte Chimoattractant Protein 1 (CCL2)
MEB : Microscopie Electronique à Balayage
MESH : Medical Subject Headings
MET : Microscopie Electronique à Transmission
MMP : Matrix Métallo-Protéinases
MOI : Multiplicity Of Infection

MOOC : Massive Online Open Course
MRM : MultiReview Manager
NAC : N-Acétyl Cystéine
NO: Monoxyde d'azote
NOD : Nucleotide-binding oligomerization domain receptors
OMS : Organisation Mondiale de la Santé
OPN : Ostéopontin
OPG : Ostéoprotégérine
PAMP : Pathogen Associated Molecular Pattern
PBS : Phosphate Buffer Saline
PEC : Polymères Extra-Cellulaires
PET : Polytéréphtalate d'éthylène
PGE2: Prostaglandin E2
RANK-RANKL : Receptor Activator of Nuclear kB et son ligand
RLO : Radicaux Libres Oxygénés
SDF : Stromal Derived Factor
SOD: SuperOxyde Dismutase
SSC : Side Scatter
SVF : Sérum de Veau Fœtal
SVF : Stromal Vascular Fraction
TGF : Transforming Growth Factor
TIMP : Inhibiteur de Métallo-protéinases
TNF : Tumor Necrosis Factor
TLR : Toll-like Receptor
UCMSC : Umbilical Cord Mesenchymal Stromal Cell
UFC: Unité Formant des Colonies
VEGF : Vascular Endothelial Growth Factor
XML: eXtensible Markup Language

Introduction

Le déluge actuel de données est un enjeu majeur de notre société, tant sur le plan technique, technologique que politique, scientifique et écologique.

Le futur des sciences biomédicales doit intégrer l'analyse des grands fichiers de données. Il s'agit d'exploiter des résultats déjà publiés pour générer de la nouvelle connaissance ; c'est un moyen d'optimiser les ressources scientifiques disponibles. La méthodologie doit être aussi rigoureuse que pour les autres types de recherche, elle se base sur le concept du « knowledge discovery » ou processus de découverte des données et peut être complémentaire de la méthodologie des revues systématiques. Cette révolution doit s'accompagner de l'aide d'ingénieurs informatiques et/ou de personnes formées en bio-informatique pour manipuler de manière efficiente les données.

Les éditeurs de journaux biomédicaux encouragent de plus en plus les auteurs à enregistrer leurs essais cliniques dans des registres dédiés, avant leur démarrage. L'exploration de ces registres est donc un reflet intéressant de la dynamique de ces essais : en effet, l'analyse permet l'anticipation, révélant une direction qui peut se concrétiser par des publications jusqu'à plusieurs années après. Il est ainsi possible d'identifier de manière précoce les tendances, les manques dans la connaissance, et de faire évoluer concomitamment préclinique et clinique. Ainsi, un enregistrement d'étude apporte en lui-même une information, alors même que sa publication finale pourrait ne pas voir le jour faute de résultat significatif (biais de publication).

La première partie de ce travail introduit un nouveau concept d'analyse des enregistrements des essais cliniques et de la dynamique de leur évolution, aussi bien thématique que temporelle. En guise d'exemple, ce concept a été tout d'abord appliqué à la médecine régénérative, démontrant de manière flagrante, ce dont beaucoup avaient l'intuition : l'absence de rationnel physiopathologique dans l'utilisation actuelle des cellules souches.

Parmi l'ensemble des thématiques abordées en médecine régénérative par cellules souches, les pathologies odonto-stomatologiques sont très peu étudiées par des essais cliniques, malgré la nécessité de prendre en charge les parodontites, pathologies immuno-infectieuses responsables de la destruction du tissu de soutien des dents. La deuxième partie de ce travail a donc souligné l'enjeu majeur du soin de ces affections, du fait de l'association potentielle avec 57 pathologies

systémiques ; le registre des essais cliniques de l'Organisation Mondiale de la Santé ayant été analysé. Bien que les auteurs s'accordent sur la responsabilité de l'écologie immunitaire et microbienne dans la physiopathologie de la maladie, les raisons de la dysbiose, de la susceptibilité individuelle sont encore mal connues. De plus, certains patients échappent à la stabilisation de la pathologie, quels que soient les thérapeutiques, qui donnent encore des résultats peu prédictibles. Compte tenu des progrès de la médecine régénérative, il est suggéré que l'utilisation des cellules stromales mésenchymateuses (CSM) permettrait de traiter efficacement les parodontites. Les CSM sont isolables à partir de la plupart des organes, les plus communément utilisées étant celles issues de la moelle osseuse, du cordon ombilical et du tissu adipeux (ASC). Les ASC représentent une source cellulaire prometteuse pour une utilisation en thérapie parodontale, pour de nombreuses raisons de sécurité, d'ergonomie de prélèvement et de culture.

L'efficacité et la sureté de l'utilisation des CSM et en particulier des ASC en régénération parodontale ont été démontrées dans des modèles animaux (bien qu'encore faiblement représentatifs de la physiopathologie de la maladie). Nous faisons l'hypothèse que les ASC constituent une source cellulaire à l'ergonomie adaptée à la physiopathologie des parodontites, permettant une prise en charge de la régénération tissulaire dans un milieu d'infection et d'inflammation résiduelles, afin de rendre l'environnement favorable à l'activation et l'action des progéniteurs endogènes (retour à l'homéostasie). Cette deuxième partie apporte donc des données quant à l'efficacité des ASC pour améliorer de manière quantitative et qualitative la régénération des tissus de soutien parodontaux dans un modèle murin original développé dans nos laboratoires, où les lésions parodontales ont été générées par l'administration de bactéries parodonto-pathogènes. Nous exposons également un protocole d'étude expérimentale qui démontre un effet antibactérien à large spectre des ASCs.

Partie I
Big Data, Big Science & Stem Cells

Table des illustrations

I. Big Data, Big Science & Stem Cells

I.1 Les enjeux de « Big Data »

I.1.1 Définition

« Entre les débuts de la culture humaine et 2003, l'humanité a produit 5 exa-octets d'informations [soit 5 millions de téraoctets]. Aujourd'hui nous produisons autant d'information tous les deux jours ».

Éric Schmidt (Directeur général de Google), Davos 2010

Cette constatation rend compte de l'ampleur de la production de données numériques dans notre société. Avec ces chiffres vertigineux, nous nous acheminons vers la production du gogol d'informations (10^{100} octets) par jour. Comme le développe le Pr Serge Miranda (Université Nice Sophia Antipolis) dans le MOOC (Massive Online Open Course) « BD**2 Des Bases de Données à Big Data », leurs sources sont multiples, provenant de données accessibles librement (ex. données gouvernementales), de données en temps réel (ex. objets connectés, réseaux sociaux) et dont la forme est plus ou moins structurée (par exemple les données provenant de forums ne sont pas structurées).

La définition *stricto sensu* de « Big Data » ou masse de données est extrêmement variable. Une des manières d'appréhender ce concept est de parler des [4V], définition du Pr Michaël Stonebraker (du laboratoire des sciences informatiques et de l'intelligence artificielle au MIT) modifiée par Alex Popescu (architecte logiciel). Il s'agit d'exprimer le [Volume] des données, actuellement de l'ordre du téraoctet au pétaoctet. Il est nécessaire d'anticiper la rapidité de traitement de l'information qui arrive en flux continu (déluge de données), [Velocity]. Nous sommes également confrontés à une grande [Variété] en terme de contenus : différentes sources de données, des structurations différentes, des contenus hétérogènes. Enfin, la [Variabilité] en terme d'évolutivité des données et de flexibilité de traitement et d'analyse, doit être considérée.

Information n'est néanmoins pas connaissance. L'information s'enrichit lorsqu'elle est partagée ; mais la connaissance est de nature individuelle, dans un contexte social donné et s'échange plus difficilement (1). Ainsi, le nouvel enjeu face à ces données est de favoriser l'interdisciplinarité en agrégeant des responsables métier, des informaticiens, mathématiciens… Comme nous l'envisagerons et le détaillerons dans le domaine de la santé,

la méta-information, c'est-à-dire les informations qui sont rajoutées pour nous permettre de comprendre et de décoder l'information, est également fondamentale (1).

I.1.2 Les lobbies industriels

Nous assistons aujourd'hui à une guerre économique des grands volumes de données que tentent de se partager les entreprises de l'EGAFA (Ebay, Google, Apple, Facebook, Amazon). La firme à la pomme a annoncé la publication d'un logiciel « open source », ResearchKit dont l'ambition est de faciliter la recherche dans le monde de la santé. De nombreuses « apps » ont été mises en ligne par des institutions comme Stanford Medicine ou le Massachusetts General Hospital, qui ont pour objectif à la fois d'apporter des informations aux patients (éducation, auto surveillance) et de faciliter la recherche sur l'asthme, le cancer du sein, les maladies cardio-vasculaires, le diabète ou encore la maladie de Parkinson. L'arrivée sur le marché de l'Apple Watch et de ses capteurs biométriques devrait permettre une montée en charge de ces dispositifs (Communiqué de Presse Apple 09/03/2015), ce qui restera toutefois à confirmer.

Le géant Google se lance également dans la médecine personnalisée, et s'associe à Novartis pour mettre au point des lentilles connectées, permettant de mesurer la glycémie dans les larmes (Communiqué de Presse Novartis 15/07/2014). Pour 99 dollars, 23andme.com se propose de réaliser une analyse de notre ADN, nous proposant « d'en savoir plus à propos de nous et de notre famille ». Cette firme met à disposition une cartographie de nos risques génétiques (i.e. risque de cancer colorectal, d'infarctus du myocarde). D'autres sociétés se sont engouffrées dans le créneau. Labgenetics (proposant les mêmes services), implanté à Madrid et dominant le secteur européen, enregistrait une augmentation de résultats de 40% en 2006.

Que deviennent ces données et à quoi serviront-elles à l'avenir ? La médecine génomique peut sauver des vies, en permettant par exemple de diagnostiquer certaines mutations spécifiques tumorales et la mise en œuvre de thérapeutiques ciblées (2). Mais la récupération des données par les compagnies d'assurance, en particulier d'assurance vie, pose le débat. En France, le code des assurances interdit aux compagnies d'utiliser les résultats des tests génétiques, même si les résultats ont été fournis par l'assuré en personne. Pourtant dans d'autres pays d'Europe, cette pratique est autorisée comme en Suisse, aux Pays-Bas ou au Royaume-Uni (3). Aux Etats-Unis, aucune décision n'a encore été prise (4). Le déluge de données que connait notre société implique donc une réflexion éthique poussée quant aux dérives potentielles de leur utilisation

(5, 6). La position de la France doit être ferme pour lutter contre le lobbying assurantiel : ces données doivent faire l'objet d'un droit à l'oubli.

I.2 Les grands volumes de données publiques en sciences médicales

I.2.1 Les futurs enjeux de ces bases de données

Si les industriels s'engouffrent dans ce secteur de marché, les organismes publics ont réellement pris conscience de l'importance de ce phénomène. En France, et depuis plusieurs années, les financements de l'Agence Nationale pour la Recherche, flèchent cette problématique (Plan d'action et appel à projet générique 2015). Cet appel d'offre 2015, identifie la gestion des données massives comme « un défi sociétal, pluri et transdisciplinaire, afin d'extraire les connaissances pour comprendre et prévoir ». A un niveau plus local, dans une réflexion sur la recherche en soin courant, le CHU de Toulouse fait le constat qu'en 2012 le volume de production de données était de 40To/an, alors qu'en 2020, il sera de 600To/an. Le CHU envisage également la notion de coût, variant de 1.000 à 48.000 €/To suivant le niveau de prestation demandé (7). Ainsi sont mis en exergue les futurs enjeux de stockage, de partage et d'intégration des informations.

Ces nouvelles règles dictées par l'économie numérique imposent également une vraie réflexion au niveau juridique et éthique car un réexamen des données peut amener à des découvertes fortuites des années après, notamment lorsque l'examen n'avait pas été réalisé dans cet objectif. Cette problématique se retrouve particulièrement en radiologie, et amène à parler « d'incidentalome » (8).

A l'heure où les financements publics et privés s'amenuisent, les opportunités scientifiques, le champ d'investigation encore libre des recherches, n'ont jamais été aussi importants (9, 10). Ce paradoxe associé à l'augmentation croissante des coûts, implique de développer une nouvelle façon de faire de la recherche, en exploitant des données locales ou publiques. Tous les domaines médicaux produisent du « Big Data » : le soin courant, la recherche clinique, la recherche fondamentale. Weber *et al.* (11) fait la démonstration de la variété des données structurées ou semi-structurées en sciences médicales. En recherche clinique, des méta-analyses des études sont réalisées (12) ; il est encore plus puissant de réaliser des méta-analyses en données individuelles (13). Un cytomètre en flux enregistre des milliers d'informations à la seconde, les puces génétiques évaluent plusieurs centaines, milliers de gènes à la fois… Nous

n'exploitons pas encore complètement toute « l'omique » : génomique, protéomique, transcriptomique, métabolomique, microbiome et intéractome (interactions protéines/protéines)(14). Du fait du prix important de ces techniques, et de la difficulté de conclure à partir d'un seul jeu de données, la mise en commun des données de plusieurs laboratoires est donc un enjeu (15).

Ainsi, l'analyse informatique et non biaisée de ces données, de manière contrôlée, pourrait être cruciale pour identifier de nouveaux espaces pertinents de recherche et tester de nouvelles hypothèses (11, 16).

Nous sommes cependant encore limités par notre capacité d'exploitation de ces données (16) et par la difficulté de leur mise en commun. Les masses d'informations à traiter dépassent notre capacité à les traiter « à la main ». Plus leur volume grossira, plus le nombre d'ordinateurs nécessaires pour traiter les données augmentera. Mettre en commun les ressources implique aussi l'interopérabilité, c'est-à-dire notre capacité que deux informations de même nature, structurées différemment, puissent être compilées et exploitées. Nous reviendrons plus largement sur cette notion dans le paragraphe suivant. Voilà des futurs enjeux de la révolution biologique numérique.

I.2.2 Structuration

Le partage et l'agrégation des données impliquent l'interopérabilité. L'interopérabilité est définie par le Larousse comme « la capacité de matériels, de logiciels ou de protocoles différents à fonctionner ensemble et à partager des informations ». Le monde médical ne diffère pas d'autres secteurs professionnels par l'individualisation d'une interopérabilité syntaxique (technique), sémantique et l'intégration de ces systèmes au quotidien (17, 18). Ainsi par cette définition, l'exploitation des données médicales nécessite :

1) de connaitre la manière dont les données sont structurées (syntaxe),
2) de se baser sur un vocabulaire commun et reconnu permettant l'analyse (sémantique) et
3) d'avoir une analyse critique des résultats (intégration).

Dans le domaine syntaxique, XML est un langage permettant de distinguer la structure et l'allure d'un document. Il se base sur l'utilisation de balises personnalisées, structurant les messages, repérant l'information (17, 19). L'ensemble des balises autorisées pour une classe de documents est défini dans une DTD (*Document Type Definition*). Nous pouvons ainsi imaginer

des balises identifiant le nom, prénom et numéro de sécurité sociale des patients, aussi bien que des balises spécifiant la mise en forme du texte. La complexité médicale peut s'exprimer à travers le langage XML, permettant même de passer d'un format aplati (organisation hiérarchique des balises) à une reconstitution relationnelle des données (19)(Figure I-1). Nous pouvons donner en exemple la mesure de la fréquence cardiaque d'un patient ; celle-ci est dépendante du contexte (l'endroit du corps où la mesure a été faite, le moment de la journée, le type de matériel).

Pour illustrer la puissance d'XML pour l'échange d'informations médicales, citons le projet HL7, fondé en 1987 (Health Level Seven International, http://www.hl7.org/), qui est un standard basé sur un format définissant l'échange, l'intégration, le partage et la réception des informations médicales de manière électronique. XML peut représenter le modèle HL7 v3 (20).

Ainsi, nous faisons la distinction entre les données proprement dites et leur contexte, leur structure, leur description (métadonnées). Le domaine de la santé nécessite d'avoir des systèmes de classification, d'ontologie, lui permettant d'organiser ses informations, de donner la possibilité aux utilisateurs de mettre en place des requêtes, d'effectuer des recherches de manière systématique, objective et reproductible. Respecter la synthase *ad hoc* d'un document, avoir des normes, des standards, signifie assurer la communication sur le long terme.

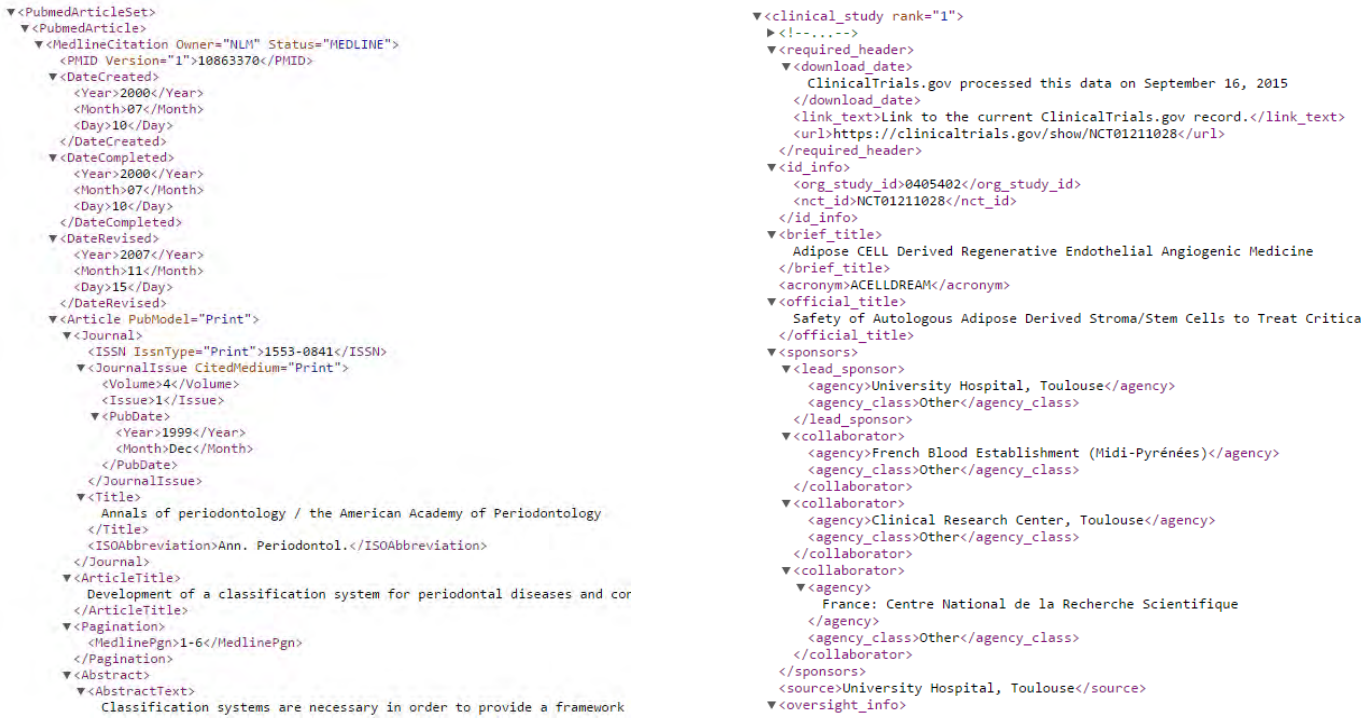


Figure I-1 : exemple de structuration XML issue de Pubmed (à gauche) et de ClinicalTrials.gov (à droite). Les différences de structuration XML peuvent être observées (DTD différentes).

I.2.3 Base de données PubMed

Base de données emblématique, PubMed contient les citations issues des journaux des sciences de la vie avec pour la plupart résumés et liens vers le texte intégral (21). Cette base est entretenue par le NCBI (National Center for Biotechnology Information) et contient environ 24 millions de citations. NCBI implémente également d'autres bases de données, par exemple dans le domaine des génotypes et phénotypes (dbGAP), des composés chimiques (PubChem), des données d'essais cliniques (BioProject)(21). NCBI met donc à disposition des chercheurs des données, téléchargeables, documentées en terme de structure XML, et permettant la mise en place d'interfaces pour faciliter leur traitement, agrégation et analyse par des outils informatiques.

Le MeSH® constitue un système de vocabulaire contrôlé dans le domaine de la santé. Cette ontologie contient des titres principaux (« mesh headings ou MH »), des sous-titres (« sub headings »), des termes de concepts supplémentaires (« supplementary concept terms ») et des mots clefs (« entry terms »). Voici un exemple illustrant une telle structuration (Figure I-2). Les articles dans PubMed sont indexés à l'aide de cette ontologie. Les MH sont constitués de 16 catégories dont le champ d'exploration est extrêmement large. Ces termes sont classifiés selon des relations hiérarchiques (22). La branche C (« Diseases ») nous intéressera plus particulièrement (Figure I-3). Elle permet de classifier les pathologies humaines à différents niveaux de spécificité. De plus, le système est redondant car une pathologie peut être placée dans plusieurs catégories à la fois (22); par exemple le syndrome de Behcet (pathologie inflammatoire des vaisseaux sanguins avec manifestations au niveau des muqueuses, comme des aphtes buccaux ou génitaux ainsi que de manière variable d'autres atteintes comme les yeux et la peau) est classifié à la fois dans les pathologies stomatognathiques (C07), les pathologies oculaires (C11), les pathologies cardiaques (C14) et les pathologies de la peau et des tissus conjonctifs (C17).

L'enregistrement de chaque étude dans une base de données sera à l'avenir aussi important que sa présence en soi dans cette base, car la présence de ses métadonnées permettra de la rendre plus visible, lisible et exploitable.

MeSH Heading	Periodontitis
Tree Number	C07.465.714.533
Annotation	general; prefer specifics
Scope Note	Inflammation and loss of connective tissues supporting or surrounding the teeth. This may involve any part of the PERIODONTIUM. Periodontitis is currently classified by disease progression (CHRONIC PERIODONTITIS; AGGRESSIVE PERIODONTITIS) instead of age of onset. (From 1999 International Workshop for a Classification of Periodontal Diseases and Conditions, American Academy of Periodontology)
Entry Term	Pericementitis
See Also	Subgingival Curettage
Allowable Qualifiers	BL CF CI CL CN CO DH DI DT EC EH EM EN EP ET GE HI IM ME MI MO NU PA PC PP PS PX RA RH RI RT SU TH UR US VE VI
Online Note	use PERIODONTITIS to search PERICEMENTITIS 1969-78
History Note	65; PERICEMENTITIS was see under PERIODONTITIS 1969-78
Date of Entry	19990101
Unique ID	D010518

MeSH Tree Structures

Stomatognathic Diseases [C07]
- Mouth Diseases [C07.465]
  - Periodontal Diseases [C07.465.714]
    - Furcation Defects [C07.465.714.204]
    - Gingival Diseases [C07.465.714.258] +
    - Peri-Implantitis [C07.465.714.282]
    - Periapical Diseases [C07.465.714.306] +
    - Periodontal Atrophy [C07.465.714.354] +
    - Periodontal Cyst [C07.465.714.470]
    - ▶ Periodontitis [C07.465.714.533]
      - Aggressive Periodontitis [C07.465.714.533.161]

Figure I-2 : exemple du MeSH heading « Periodontitis ». Celui-ci est classifié dans la branche C07 « Stomatognathic Diseases », et situé dans la troisième sous-catégorie de cette branche (C07.465.714.533). Ce terme fait également référence au terme « Pericementitis ». De nombreux qualificatifs peuvent être utilisés pour décrire plus finement ce champ, comme PC (« Prevention & Control »). Capture d'écran provenant de https://www.nlm.nih.gov/mesh/2015/mesh_browser/.

1. + Anatomy [A]
2. + Organisms [B]
3. − Diseases [C]
    - Bacterial Infections and Mycoses [C01] +
    - Virus Diseases [C02] +
    - Parasitic Diseases [C03] +
    - Neoplasms [C04] +
    - Musculoskeletal Diseases [C05] +
    - Digestive System Diseases [C06] +
    - Stomatognathic Diseases [C07] +
    - Respiratory Tract Diseases [C08] +
    - Otorhinolaryngologic Diseases [C09] +
    - Nervous System Diseases [C10] +
    - Eye Diseases [C11] +
    - Male Urogenital Diseases [C12] +
    - Female Urogenital Diseases and Pregnancy Complications [C13] +
    - Cardiovascular Diseases [C14] +
    - Hemic and Lymphatic Diseases [C15] +
    - Congenital, Hereditary, and Neonatal Diseases and Abnormalities [C16] +
    - Skin and Connective Tissue Diseases [C17] +
    - Nutritional and Metabolic Diseases [C18] +
    - Endocrine System Diseases [C19] +
    - Immune System Diseases [C20] +
    - Disorders of Environmental Origin [C21] +
    - Animal Diseases [C22] +
    - Pathological Conditions, Signs and Symptoms [C23] +
    - Occupational Diseases [C24] +
    - Chemically-Induced Disorders [C25] +
    - Wounds and Injuries [C26] +
4. + Chemicals and Drugs [D]
5. + Analytical, Diagnostic and Therapeutic Techniques and Equipment [E]
6. + Psychiatry and Psychology [F]
7. + Phenomena and Processes [G]
8. + Disciplines and Occupations [H]
9. + Anthropology, Education, Sociology and Social Phenomena [I]
10. + Technology, Industry, Agriculture [J]
11. + Humanities [K]
12. + Information Science [L]
13. + Named Groups [M]
14. + Health Care [N]
15. + Publication Characteristics [V]
16. + Geographicals [Z]

Figure I-3 : Structuration du MeSH, avec le détail de la branche C « Diseases »

I.2.4 Base de données ClinicalTrials.gov

Sous la responsabilité du « National Library of Medicine (NLM) » et du « National Institutes of Health (NIH) », « ClinicalTrials.gov (CTD) » est une base de données d'enregistrement des essais cliniques. Celui-ci contient environ 190.000 études, représente environ 190 pays et les

50 états des USA ; 30% des études viennent d'Europe et 50% des USA. Ce n'est d'ailleurs pas le seul registre puisque l'OMS est en charge de « l'International Clinical Trials Registry Platform (ICTRP) ». Néanmoins, CTD en forme 60-80% des résultats. Les enregistrements de CTD sont précis, avec des interventions détaillées, et 24 champs sont conformes aux exigences de « l'International Committee of Medical Journal Editors » et de l'ICTRP. Des descripteurs MeSH pour les conditions sont générés par un algorithme, et toutes les données (y compris les résultats éventuels publiés) sont exportables au format XML. Le risque de mauvaise classification a été évalué, variable selon la spécialité clinique, mais avoisine seulement les 3% d'études (23). Certains enregistrements reflètent même mieux la réalité que les publications finales (24).

Il s'agit donc d'un registre représentatif, puissant, source de données fiables.

Néanmoins, la mise en place d'un système de surveillance pérenne de la littérature basé sur ces registres nécessite quand même des financements afin d'effectuer des mises à jour, et de mettre en place des évolutions (25).

I.2.5 Le processus de découverte de données

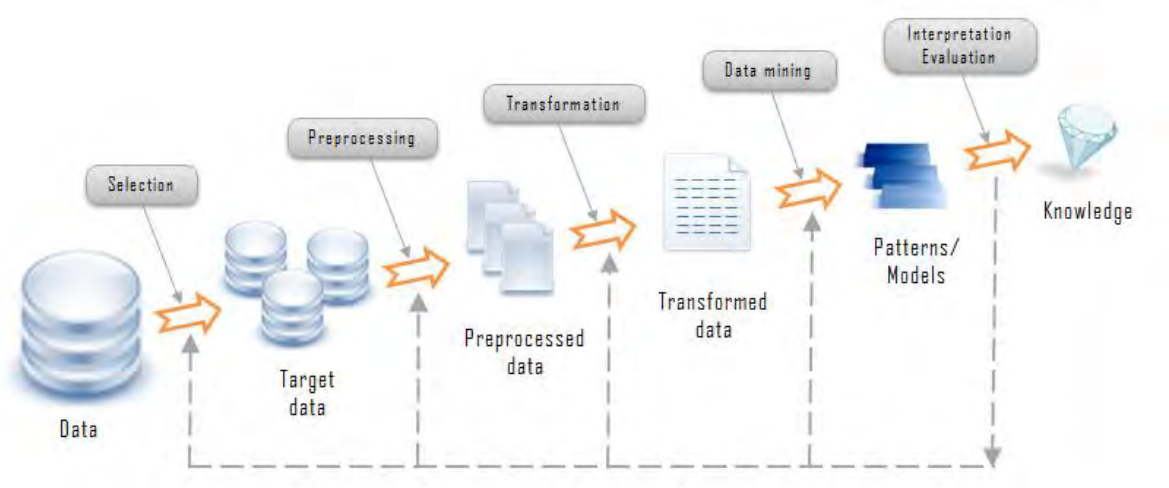


Figure I-4 : Processus de découverte de données, les différentes étapes. Les données sont sélectionnées, nettoyées, réduites, et explorées après formulation d'hypothèses. L'interprétation de ces données permet à nouveau de définir de nouvelles hypothèses et de faire évoluer la connaissance. Schéma emprunté de http://www.rithme.eu/?m=resources&p=kdprocess&lang=en et de (26).

Grâce aux éléments détaillés précédemment, nous pouvons mettre en place le processus de découverte de connaissances dans les bases de données (« Knowledge Discovery in Databases ou KDD process (26) »). Ce processus est illustré par la figure ci-dessus (Figure I-4). Celui-ci peut être artificiellement découpé en différentes étapes séquentielles:

1. L'objectif de recherche de départ doit être clairement posé.

2. Sélection des données: il est nécessaire de travailler avec des bases de données pertinentes, en adéquation avec la question de recherche, accessibles facilement (comme des bases de données publiques), mises à jour régulièrement avec des niveaux bas d'erreurs et de mauvaise classification (26).

3. Nettoyage des données et prétraitement : En faisant l'analogie avec le domaine des signaux électromagnétiques, il est nécessaire d'optimiser le rapport signal sur bruit. Ceci peut être réalisé en prenant des décisions quant aux données manquantes.

4. Réduction des données et projection : à partir du jeu global de données, un sous-ensemble peut-être étudié, des variables peuvent être fusionnées.

5. Analyse exploratoire, modèle et hypothèse : en fonction de l'objectif de départ, du jeu de données obtenu au final, la méthode d'exploration de données (« data mining ») peut-être choisie. Ces méthodes peuvent être différentes en fonction des différentes parties de l'hypothèse.

6. Exploration des données proprement dites : en fonction de la technique choisie (i.e. regroupements ou « clustering », arbres de classification, méthodes de régression). Nous sommes alors à la recherche de motifs, de modèles particuliers, de signatures particulières.

7. Interprétation des données : la manière dont sont représentées les données est importante, car l'aide visuelle aide au processus de découverte des données, permet de mettre en évidence les manques dans la connaissance, et apporte un impact significatif pour délivrer des messages, en particulier en biologie (27). L'utilisation d'arbres phylogénétiques est notamment intéressante pour représenter des relations de parenté entre éléments ; un

thésaurus peut donc être visualisé de cette manière. Il est alors possible de retourner aux étapes de 1 à 6 autant de fois que cela s'avère nécessaire.

8. Agir sur les connaissances : ces connaissances seront utilisées pour interpréter et développer de nouveaux concepts (28, 29). Lorsque ces étapes sont renouvelées régulièrement, leur automatisation (autant que faire se peut) est particulièrement utile pour la prise de décision politique en santé (23).

Analyser des données obéit donc à des règles scientifiques aussi strictes que celles d'un protocole de biologie. La procédure est garante de l'intégrité de l'analyse, pouvant être appliquée à n'importe quel domaine de la connaissance. Dans la suite de ce travail, il nous a semblé intéressant et novateur d'utiliser une telle procédure pour envisager les différents aspects de la médecine régénérative par cellules souches. Comme nous le détaillerons ci-après, l'analyse est issue de la base publique ClinicalTrials.gov, avec une sémantique basée sur le thésaurus MeSH.

I.3 Cellules souches et médecine régénérative

I.3.1 Article 1: "An innovative, comprehensive mapping and multi-scale analysis of registered trials for stem cell based regenerative medicine"

Le travail présenté est accepté pour publication dans le journal « Stem Cells Translational Medicine ».

Contexte

Sous le vocable de cellules souches se cachent de nombreux types cellulaires et définitions. Tout d'abord nous pouvons distinguer les cellules embryonnaires (Embryonic Stem Cells ESC), totipotentes lorsqu'elles dérivent de l'oocyste fécondé, ou pluripotentes lorsqu'elles dérivent de la masse interne du blastocyste (30), à l'origine de l'embryon et non de ses annexes. Ces cellules vont se spécialiser pour former les cellules de chaque couche embryonnaire primitive (ectoderme, mésoderme et endoderme). La recherche sur les cellules embryonnaires en est encore à ses débuts car leur isolation chez l'Homme ne date que des années 1998 (31).

Comparativement, sont décrites des cellules souches adultes. Leur rôle est de maintenir et de réparer les tissus dans lesquels elles sont retrouvées. La preuve de concept de l'utilisation des cellules souches adultes a été largement démontrée à travers la greffe de moelle osseuse (32, 33). Les travaux sur les cellules souches hématopoïétiques (HSCs) ont également permis de mettre en évidence un autre type cellulaire dans la niche médullaire : les cellules souches mésenchymateuses (CSMs) à l'origine des cellules stromales dans la moelle. Les CSMs peuvent être potentiellement isolées de n'importe quel organe. Tout ceci peut expliquer l'engouement majeur et l'explosion des essais cliniques utilisant des CSMs (34-36), basés sur les effets paracrines des CSMs (dont effet immunomodulateur) plus que sur la transdifferenciation et le remplacement des cellules endommagées (37).

Dans ce domaine encore jeune de la thérapie cellulaire, l'augmentation importante des données précliniques et cliniques rend difficile la vision globale des tendances et des futures directions à prendre (38, 39). Nous avons choisi d'étudier les enregistrements des essais cliniques utilisant des cellules souches à l'aide du registre ClinicalTrials.gov (CTD). Particulièrement bien structuré, ce registre est le plus adéquat pour effectuer des agrégations et des analyses de données. Que les études soient publiées ou non, en cours de recrutement ou terminées,

l'exploration du registre permet d'obtenir un cliché représentatif et actuel de la complexité du champ.

L'objectif de cette étude est de cartographier l'ensemble des essais cliniques enregistrés sur ClinicalTrials.gov utilisant la thérapie cellulaire par cellules souches, d'explorer la diversité et la temporalité de la mise en place des champs d'application, en utilisant diagrammes de chorde et arbre phylogénétique pour visualiser et analyser les données.

Méthodologie

Notre stratégie de recherche se base sur les mots clefs « mesenchymal OR stromal OR stem OR progenitor » et la dernière mise à jour a été réalisée le 28/06/2015. Le thésaurus du MeSH a été utilisé pour classifier les pathologies traitées par les cellules souches, à la fois à un niveau de faible granulométrie (le niveau du champ d'application) et à forte granulométrie (le niveau de la pathologie spécifique).

Nous avons également développé un script de programmation écrit en langage « Perl », qui nous a permis de sélectionner les études facilement, et surtout de réaliser plus simplement le processus de KDD qu'à la main. En effet, en utilisant quelques lignes de programmation, nous avons pu effectuer des analyses en sous-groupes et des comparaisons croisées multiples de variables. « Perl » est un langage assez classique quant à l'utilisation des fonctionnalités de boucles (foreach), des structures conditionnelles (if/then/else), des déclarations (de variables tableaux, scalaires ou hash). Mais une de ses grandes forces est la possibilité d'utiliser les expressions rationnelles, c'est-à-dire la recherche et/ou le remplacement de chaines de caractères rapidement dans de grands jeux de données.

Résultats

Sur la totalité des 5788 entrées étudiées, 1497 (61.5%) étaient en relation avec l'utilisation des cellules souches hématopoïétiques après chimiothérapie ou radiothérapie. Nous nous sommes donc restreints aux 939 restants dont 51.1% étaient en relation avec des CSMs.

Au final, les résultats ont révélé un manque de stratégie globale quant à l'utilisation des cellules souches, sans réelle spécificité d'un type cellulaire vis-à-vis d'une pathologie spécifique.

Nous montrons ainsi que les études utilisant des CSMs de manière allogénique constituent la moitié des études sur les CSMs, et reçoivent plus de financements industriels que l'autologue ($p<0.001$). Au fur et à mesure du temps, l'utilisation des cellules cultivées a grandement augmenté, particulièrement depuis 2009. Les cellules issues du tissu adipeux sont de plus en plus utilisées comparativement à la moelle osseuse. Les ASCs sont utilisées de manière prépondérante en autologue ($p<0.001$), dans les pays Européens ($p<0.01$) et par l'industrie ($p=0.02$) par rapport aux autres CSMs. Les détails pour chaque mot clef MeSH sont disponibles en ligne sur http://multireview.perso.sfr.fr/.

Article

An innovative, comprehensive mapping and multi-scale analysis of registered trials for stem cell based regenerative medicine

Journal:	*Stem Cells Translational Medicine*
Manuscript ID	SCTM-15-0329.R1
Manuscript Type:	Original Research
Date Submitted by the Author:	05-Jan-2016
Complete List of Authors:	Monsarrat, Paul; Paul Sabatier University, Dental Faculty, Department of Anatomical Sciences and Radiology, Toulouse University Hospital, Toulouse, France.; CNRS 5273; UMR STROMALab; University of Toulouse UPS; INSERM U1031; EFS Pyrenees – Mediterranean; Toulouse, France. Vergnes, Jean-Noel; Paul Sabatier University, Dental Faculty, Department of Epidemiology and Public Health, Toulouse University Hospital, Toulouse, France.; Division of Oral Health and Society, Faculty of dentistry, McGill University, Montreal, Quebec, Canada. Planat-Benard, Valerie; University Paul Sabatier, UMR 5241 CNRS Ravaud, Philippe; Paris Descartes University, Faculty of Medicine, Paris, France; INSERM UMR-1153, Paris, France; Department of Epidemiology, Columbia University Mailman School of Public Health, New York, NY, USA Kémoun, Philippe; Paul Sabatier University, Dental Faculty, Department of Biological Sciences, Toulouse University Hospital, Toulouse, France. Sensebe, Luc; CNRS 5273; UMR STROMALab; University of Toulouse UPS; INSERM U1031; EFS Pyrenees – Mediterranean; Toulouse, France. Casteilla, Louis; UMR UPS/CNRS/EFS 5273 Inserm U1031 , STROMALab
Keywords:	Mesenchymal Stromal Cells, Stem cells, Clinical Trials as Topic, Data Mining, Regenerative Medicine

An innovative, comprehensive mapping and multi-scale analysis of registered trials for stem cell based regenerative medicine

Subtitle: Mapping of stem cell based registered trials

Authors

Paul Monsarrat [1], Jean-Noel Vergnes [2], Valérie Planat-Bénard [3], Philippe Ravaud [4], Philippe Kémoun [5], Luc Sensebé [6], Louis Casteilla [7]

Affiliations

1: Paul Monsarrat, DDS (corresponding author)
Paul Sabatier University, Dental Faculty, Department of Anatomical Sciences and Radiology, Toulouse University Hospital, Toulouse, France.
&
CNRS 5273; UMR STROMALab; University of Toulouse UPS; INSERM U1031; EFS Pyrenees – Mediterranean; Toulouse, France.

Mail address: UFR Odontologie de Toulouse
3, chemin des maraîchers
31062 Toulouse Cedex 9.
E-mail address: paul.monsarrat@gmail.com
Telephone number: +33 6 84 47 48 55
Fax number: +33 5 61 25 47 19

2: Jean-Noel Vergnes, PhD, DDS
Paul Sabatier University, Dental Faculty, Department of Epidemiology and Public Health, Toulouse University Hospital, Toulouse, France.
&
Division of Oral Health and Society, Faculty of dentistry, McGill University, Montreal, Quebec, Canada.
E-mail address: jn.vergnes@mcgill.ca

3: Valérie Planat-Bénard, PhD

CNRS 5273; UMR STROMALab; University of Toulouse UPS; INSERM U1031; EFS Pyrenees – Mediterranean; Toulouse, France.
E-mail address: valerie.planat@inserm.fr

4: Philippe Ravaud, PhD, MD
Paris Descartes University, Faculty of Medicine, Paris, France
&
INSERM UMR-1153, Paris, France
&
Department of Epidemiology, Columbia University Mailman School of Public Health, New York, NY, USA
E-mail address: philipperavaud@gmail.com

5: Philippe Kémoun, PhD, DDS
Paul Sabatier University, Dental Faculty, Department of Biological Sciences, Toulouse University Hospital, Toulouse, France.
E-mail address: philippe.kemoun@univ-tlse3.fr

6: Luc Sensebé, PhD, MD
CNRS 5273; UMR STROMALab; University of Toulouse UPS; INSERM U1031; EFS Pyrenees – Mediterranean; Toulouse, France.
E-mail address: luc.sensebe@efs.sante.fr

7: Louis Casteilla, PhD
CNRS 5273; UMR STROMALab; University of Toulouse UPS; INSERM U1031; EFS Pyrenees – Mediterranean; Toulouse, France.
E-mail address: louis.casteilla@inserm.fr

Author contributions

Paul Monsarrat: Conception of design, collection and assembling the data, data analysis and interpretation, manuscript writing

Jean-Noel Vergnes: Conception of design, data analysis and interpretation, manuscript writing

Valérie Planat-Bénard: Conception and design, manuscript writing, final approval of manuscript

Philippe Ravaud: Conception of design, final approval of manuscript

Philippe Kémoun: Conception and design, manuscript writing, final approval of manuscript

Luc Sensebé: Data analysis and interpretation, manuscript writing, final approval of manuscript, language checking

Louis Casteilla: Conception and design, financial support, data analysis and interpretation, manuscript writing, final approval of manuscript, language checking

Acknowledgements

This study was supported by funding from the Midi-Pyrenees region, Paul Sabatier University and the research platform from Toulouse Dental Faculty. The authors thank Susan Becker for proofreading. We declare no conflicts of interest.

Keywords

Mesenchymal Stromal Cells, Stem Cells, Regenerative Medicine, Data Mining, Clinical Trials as Topic

Word count: 4196

Abstract

Background

We aim to provide innovative, comprehensive way of mapping the profusion of stem cell based clinical trials registered at ClinicalTrials.gov in order to explore the diversity of the fields of application and the temporal complexity of the domain, using a chord diagram and phylogenetic-like tree visualizations to help data-mining and knowledge discovery.

Methods

Search strategy: "stromal OR stem OR mesenchymal OR progenitor". The MeSH thesaurus was used to classify diseases treated by stem cells in greater depth, from large fields of application to specific diseases.

Results

Of the 5788 trials screened, 939 were included, 51.1% of which were related to MSCs. No real specificity emerged as to the therapeutic uses of the different types of stem cells. More than half the MSC studies concerned allogeneic MSCs, and received more support from industry than autologous MSC studies ($p<0.001$). Over time, the uses of cultured cells has increased greatly, particularly from 2009. Adipose tissue derived cells are also increasingly used in trials compared to bone marrow cells. The use of adipose-derived stromal cells was predominantly autologous ($p<0.001$), restricted to European countries ($p<0.01$) and supported by industry ($p=0.02$) compared to other MSCs. Details about MeSH keywords at http://multireview.perso.sfr.fr/.

Conclusion

Mapping may reveal a lack of global strategy despite the regulations and the related costs associated with good manufacturing practices. A systematic approach to preclinical data, intended to objectively and robustly reveal the most appropriate fields with the most efficient cells, is needed. Repeated exchanges between the bench and the bedside are necessary.

Introduction

In recent decades, many advances have been made in the area of stem cell therapies, revolutionizing our knowledge of tissue development, function, and physiopathology, as analyzed by the NIH [1, 2]. In fact, the broad development of bone marrow transplantation serves as the proof of concept for the use of adult stem cells [3, 4]. This demonstration also highlighted another immature cell closely associated with hematopoietic stem cells (HSC) through its niche: the mesenchymal stem cell (MSC). First discovered by Friedenstein in the 1970s [5], MSCs are multipotent but also play a key supportive role via the secretion of numerous paracrine factors including immunosuppressive molecules [6]. The identification of stem cells in almost all tissues [7] has paved the way for a large quantities of pre-clinical data and numerous clinical trials - to such an extent that it has become difficult to have a global view of current trends and future directions in this still young and continuously growing field of research [8, 9]. Heterogeneous cell populations sometimes characterized as "stem cells" (e.g. mononuclear cells from bone marrow, BMMNC, and stromal vascular fraction from adipose tissue, SVF) or the adherent and expanded counterpart that corresponds to MSCs (BM-MSCs and ASCs, respectively), are used at clinical level. Beside adult stem cells, embryonic stem cells (ESC) and induced pluripotent stem cells have given rise to great hopes and are just starting to be investigated at clinical level [10]. Many doubts about the efficacy and safety of cell therapy lead to the need for exhaustive searches of clinical trials [11], critical analysis of their methodology (i.e. risk of bias [12]) and investigation of the various parameters taken into account. The comparison between the different sources of stem cells, the utilization of heterogeneous or purified cells, and the recourse to allogeneic or autologous cell sources, are among the many issues that remain unresolved [13, 14].

Their scientific and economic implications and the associated health care policy decisions make these issues crucial.

Public availability of mass data [15, 16] opens the way to the creation of new, practical knowledge that will help physicians, researchers and policymakers to identify pertinent areas of intervention [15, 17] and reveal unexpected facts leading to the development of new concepts [18, 19]. We are keen to provide a view of the stem cell area that is both broad and deep by means of a systematic mapping review methodology [18, 20], which could help to interpret scientific information while enlarging the field of exploration [21] and providing adequate data visualization [22] at the same the time. One requirement for knowledge discovery in databases (KDD [23]) is to work with relevant, up-to-date databases associated with low noise and errors [23]. Different sources of data can be used. Analyses of published studies take advantage of the scientific peer-review process but publication is often delayed and negative trials are less likely to be published. This leads to a retrospective, and potentially biased, point of view. During the 1990s, clinical trial registers were strongly promoted in biomedical research, with the aim of revealing the existence of all trials and eliminating publication bias [24]. The International Committee of Medical Journal Editors (ICMJE) required registration of all trials starting enrollment after July 1, 2005 and ongoing clinical trials that began enrolling patients before that date [24]. Exploration of these registers gives a more up-to-date and representative snapshot of the complexity of this young and constantly evolving field of stem cells [25, 26]. Among the different registers, the ClinicalTrials.gov database (CTD) is one of the best designed for aggregation and analyses [27]. Launched in 2000, concomitantly with the setting-up of stem cell related trials, this worldwide register contains detailed, standardized characteristics of each trial, particularly keywords describing disease conditions using the MeSH thesaurus, as furnished by the U.S. National Library of Medicine. This provides an in-depth classification and enables

computer tools to be used for interpretation at both low (fields of application) and high (specific diseases) granularity.

From this database, we screened 5788 trials and, using computer tools, comprehensively mapped human stem cell based clinical trials using a chord diagram and phylogenetic-like tree visualizations. This revealed no real specificity in the uses of stem cells, including MSC, in the different fields of application. The lack of an apparent global strategy despite the regulations (e.g. requirements for advanced-therapy medicinal products and good tissue practice, in Europe and the USA, respectively [28]) and the costs associated with good manufacturing practices may be hypothesized and are probably linked to the field's strong intention to reach the market. Increasing interest in cultured and allogenic cells compared to the heterogeneous fraction and autologous uses, respectively, was revealed.

Material and methods

Data aggregation

Data aggregation was performed using a Perl script developed in the laboratory to minimize errors during the screening process and to computerize data for further analyses (Supplementary Figure 1, Supplementary Table 1).

Dataset selection, data cleaning and preprocessing

The search strategy used the keywords "stromal OR stem OR mesenchymal OR progenitor". All trials were exported into xml format, then parsed into local database. Trials were included if: 1) cell therapy was based on enriched/purified/sorted stem cells or expanded stem cells, and 2) stem cell therapy efficacy or safety was stated as an objective of the trial. For each trial included, the

original tissue for cells and the donor (autologous or allogeneic) were recorded. Only the "Diseases" branch [C] of the MeSH classification is used by the CTD to describe study conditions. This branch contains 26 sub-branches, corresponding to major disease groupings. If the CTD had failed to attribute MeSH keywords to a trial, two authors, PM and JNV, manually added the keywords they found most appropriate by consensus. The last search was performed on 2015/06/28.

Data reduction and projection

The [C23 - Pathological Conditions, Signs and Symptoms] sub-branch was removed for lack of specificity. For chord diagrams, MeSH redundancy was assumed: one trial could be classified in several fields of application. For tree rendering, the final custom MeSH structure used is described in Supplementary File 1 (selected terms in bold); desired duplicates were identified to indicate to the reader that there were several occurrences.

Exploratory analysis, model and hypothesis

The phase and size of the clinical trial, the genders of participants, the clinical trial sites, the blinding and randomization techniques employed, the type of stem cells used, the cell donors and the fields of application were described. Each CTD entry contained information about the lead sponsor and the collaborators declared by the authors, especially if this funding source was considered to derive from "NIH, Industry or Others". Using Califf et al.'s algorithm [29] on these lead sponsors and collaborators tags, the probable funding source (i.e. the main sponsor) was categorized as "NIH, Industry or Others". The start dates of trials were considered as well as their clinical phases, as markers of the progress and maturity of a theme.

Data mining

A phylogenetic-like representation was obtained using http://itol.embl.de/ [30]. Because phylogenetic trees were developed to schematically represent kinship relationships between groups of living beings, we used this type of representation to visualize relationships between diseases described in the MeSH classification. All MeSH descriptor terms extracted from included trials (disease conditions) were represented around a rooted tree. Summary statistics for each term, together with individual lists of the related trials, were provided. Internal divisions of the tree were represented by sub-levels of these terms (ancestors). Two major datasets were represented around the tree (online data): trial phases and start dates. Chord diagrams [31] were used to analyze double-entry tables (http://mkweb.bcgsc.ca/tableviewer/). We represented the proportion of trials classed in each of the MeSH sub branches (fields of application) in relationship with type of stem cells, and start dates of studies or trial phases. An example was furnished (Supplementary Figure 2). Each disease was linked to a website we generated (http://multireview.perso.sfr.fr/) where all statistics were summarized for each MeSH keyword included.

Results

A PRISMA flow diagram is given in Supplementary Figure 3. A large majority of records (1497 or 61.5%) dealt with HSC transplantation after chemotherapy or radiotherapy. We focused our analysis on the remaining 939 trials, of which 51.1% were related to MSCs (Supplementary Table 2).

Temporal evolution of stem cell use according to the applications

Ultimately, 21 MeSH fields of application were selected, allowing diseases and their consequences to be considered. Figure 1 presents the temporal evolution of these fields. Three important groups of diseases can be extracted according to their representations: (i) cardiovascular system (CVD) and nervous system diseases (NSD), (ii) musculoskeletal, immune system (ISD), digestive diseases and wounds and injuries, which really emerged from 2009, and (iii) a smaller group with nutritional, skin (and connective tissue), female urogenital (and pregnancy complications), male urogenital, endocrinal, eye and respiratory diseases. About half the studies starting in 1993-2005 dealt with CVD and their number remained quite constant over the years (about 80 studies), while NSD studies began to gain momentum from 2006 (25 studies in 2006-2008 rising to 100 in 2012-2017).

Application areas according to stem cell types (Figure 2)

We grouped cells into 4 categories: MSCs, their respective heterogeneous fractions, other hematopoietic stem cells (PBSC, EPC, CD34+ and CD133+), and other stem cells and progenitors. ESC-derived cells were considered in 10 studies, with the use of ESC-derived retinal pigment epithelial cells in 8 studies, for eye diseases. Tissue-specific stem cells were used in the corresponding application field (e.g. cardiac stem cells for CVD and corneal limbal stem cells for eye diseases). The predominant uses of EPC and CD133+ were also in CVD. Nevertheless, these cells were used in few studies, and can be considered as exceptions. A glance at the diagram shows that each cell type was concerned by multiple fields of application and, in a mirror analysis, each field of application was concerned by multiple types of cells. As a whole, Figure 2 reveals no really specific application for a specific cell type.

Description of studies using MSCs and BMMNC/SVF (Supplementary Table 2, Figure 2)

Because MSCs were involved in half the trials, we next focused on these cells. Studies using these cells enrolled predominantly small cohorts of patients (median with interquartile range [Q1; Q3] of 25 [12; 55]), 44.7% (213) of them were randomized and 67.4% of these were open-labeled. The MSCs used were allogeneic for 53% and autologous for 47%. With the website created (http://multireview.perso.sfr.fr/msc/29163.html), we found 50% of allogeneic use in CVD, but 63% and 70% for the sub-categories "Stroke" and "Myocardial Infarction".

When studies using ASCs were compared to those using other sources of MSCs, the number of enrolled patients was smaller in ASC studies (respective medians of 19 [10; 40] and 27 [12; 50], p=0.02), more uses were autologous (respectively 68.4% and 43.0% of studies, p<0.001) and activity was more restricted to European countries (respectively 55.4% and 23.3% studies, p<0.001). Concerning the main sponsor, industry supported 41.6% and 28.3% of ASC and other MSC studies respectively (p=0.02). Although the number of trials involving ASCs was smaller than for other sources of MSCs, there was no difference in study phases. ASCs were also significantly less tested in ISD (p=0.01), more tested in female urogenital diseases (p=0.04), and showed an increasing trend in digestive diseases and wounds and injuries. Nevertheless, there was no area where the ASCs were tested and other MSCs were not.

Temporal evolution of MSC applications (Figure 3)

From 1999 to 2005, 12 trials were set up in 8 fields whereas 270 trials in 20 out of 21 fields are described today. Until 2008, CVD and ISD (e.g. graft vs host, autoimmune diseases) were the most important, but their relative importance decreased over time in favor of a diversification of

the fields. NSD has increased over time to become the most studied field. Digestive and musculoskeletal investigations also developed at this time. From 2012, trials concerning respiratory and male or female urogenital diseases increased greatly, and a set of otorhinolaryngology themes emerged. Over time, allogenic MSCs became more used than autologous ones (6, 15, 81 and 144 versus 5, 31, 61 and 120 for allogenic and autologous respectively). Studies using allogeneic MSCs were also significantly more supported by industry (modest-sized companies (Supplementary Table 3)) than studies using autologous MSCs (respectively 37.0% and 22.4%, $p<0.001$).

Study phases for MSCs as a marker for maturity of the field

Phase 1-2 and 2 studies consisted of 59.6% of MSCs studies. Of 21 fields of application, only 11 fields had Phase 2-3 and 8 had Phase 3 studies registered. Digestive, then CVD, ISD and NSD fields of application appeared more mature with higher numbers of Phase 3, Phase 2-3, and Phase 2 studies (Figure 4). With 50% of Phase 1 studies, the respiratory field appeared to be a younger topic. With both a high number of Phase 1-2 and some Phase 2-3 and 3 trials, endocrine, male or female urogenital and skin diseases, and wounds and injuries seemed to be in an intermediate state of study. This tendency was confirmed with a higher ratio of Phase 1, 1/2 to Phase 2, 2/3, 3 trials for the respiratory tract and endocrine system (Figure 4, histograms). Moreover, some Phase 2 studies, e.g. for stomatognathic, respiratory or eye diseases were not represented in Phase 2-3 or Phase 3.

Expanded cells versus heterogeneous fraction and BM versus AT derived cells (Table 1, Supplementary Figure 4)

To make these comparisons, we computed different ratios. The ratio of heterogeneous fraction to expanded cells (R_S) decreased over time (R_S of 2.67 before 2006, 0.58 after 2011). This meant that heterogeneous fractions tended to be neglected in favor of cultured cells over time. Concerning study phases, heterogeneous fractions concerned more Phase 2-3/Phase 3 than other phases (R_S of 0.93 versus 0.76 and 0.60). Remarkably, 3 fields of application had lower R_S than the others, showing that cultured cells were particularly used for female urogenital, digestive and immune diseases. In the same way, R_{BM} and R_{AT} ratios compared heterogeneous versus expanded cells in BM and AT respectively. Some differences can be pointed out between these two ratios: for respiratory, male urogenital, skin and immune diseases, expanded cells were more used for BM than they were for AT. The BM to AT cells ratio ($R_{BM/AT}$) highlighted disparities between fields of application (e.g. a greater use of BM cells for CVD and ISD and a greater use of AT cells for female or male urogenital diseases compared to other fields). Despite these differences, both heterogeneous and expanded cells, and both BM and AT cells were used in all main fields of application. BM cells were also more used in Phase 3 than AT ($R_{BM/AT}$ was higher in Phase 2-3/Phase 3 than in other phases). But this ratio decreased over time ($R_{BM/AT}$ of 42.0 before 2006, 2.11 after 2011). This meant that cells from AT were increasingly used compared to BM.

Phylogenetic-like tree representation to detail uses of stem cells for each precise disease

Start dates and phases of studies were represented at the level of specific diseases, around the tree. The website (http://multireview.perso.sfr.fr/) was composed of 3 parts concerning all stem cells, MSCs and ASCs. It was created to go deeper into the architecture of the MeSH and to

obtain more details about the clinical trials using stem cells in the context of a specific disease (example for MSCs in "Cardiovascular Diseases" in Supplementary Figure 5). The following link http://itol.embl.de/shared/paulmonsarrat provides an enlargeable interactive visualization of all pathologies where stem cells are used. For instance, Supplementary Figure 6 gives a precise inventory of the pathologies that may benefit from treatment with ASCs. Again, we observed no real specificity in their uses and, even for each application, a great diversity could also be observed.

Discussion

The 2000s were marked by great interest in regenerative medicine based on adult stem cells [10, 32-35] and the establishment of financing programs before the isolation of ESCs [36]. Since this date, the increasing proliferation of pre-clinical data has been generating great hopes. The recent translation of this research to clinical trials requires an objective view of these constantly evolving fields. Our innovative new approach revealed that, apart from the classical use of hematopoietic stem cells [4], there was no strict specificity in the therapeutic uses of the different types of stem cells. We also highlighted the evolution towards the use of allogenic and cultured and expanded cells at the expense of heterogeneous and autologous fractions. Lastly, adipose tissue appears to be a tissue source that is becoming more and more privileged.

The lack of specificity is surprising with regard to the large amount of preclinical data but is symptomatic of the immaturity of this domain [6, 25, 37]. This suggests that most of what emerges at the level of clinical trials is not the logical consequence of a strong, convergent background of basic and preclinical studies but is more related to individual initiatives. This could be considered as the result of a variety of points of view. First, such therapy is thought to

treat any disease because of the pluri/multipotency and immune regulatory/suppressive action of the putative therapeutic products [38]. This is reinforced by the dream of industrial companies to have a universal cell product that treats many diseases. Second, the therapeutic products, i.e. cells, are not yet well defined and could correspond to quite different products, pluri or multipotent, heterogeneous non-purified or purified cells, non-expanded or cultured, autologous or allogenic cells with no clear comparative view as previously described. Both the above standpoints are quite different from the classic "one product for one target" approach [39]. Third, the lack of a consensual view emerging from the multiplicity of preclinical data leaves many opportunities and cell/application combinations open. This also raises questions about the physiological relevance of preclinical models to diseases. Furthermore, few preclinical and experimental studies comparing different types of cells have been conducted and published [40, 41]. This point highlights the need for a systematic approach to preclinical data to objectively and robustly reveal the most appropriate fields with the most efficient cells. Exchanges of information back and forth between the bench and the bedside are necessary. Lastly, ethical issues associated with the use of ESC in many countries [10] and the fact that opinion has often been refractory to research on ESCs [42] has led to alternative sources of therapeutic cells being found that are even less efficient.

Our analysis reveals that the cell types most often tested are MSCs, with increasing interest over time in cultured cells compared to their respective heterogeneous fraction. The cardiovascular field was the first to be clearly identified but, since 2005, the number of studies dedicated to such applications has not changed. 2005 was a key year because, from then on, all applications other than cardiovascular ones were investigated more and more. The heterogeneous fraction is accessible at the bedside within hours and is almost never characterized. In contrast, the expanded fraction needs time, and it is more expensive to comply with FDA and European

regulations [28], but this fraction can be more finely characterized and secured. The fewer important resources necessary for the heterogeneous fraction compared to expanded cells may explain why there are still more advanced phases for the former although several studies have reported modest successes or negative outcomes [43] in chronic heart failure, in contradiction with Cochrane meta-analyses [44, 45]. These elements could explain why cardiac regeneration by stem cells is no longer the main attractive area. This discrepancy in result analysis may be related to the design of the control groups [46]. In most trials, control groups did not undergo tissue sampling to isolate cell products and did not have the benefit of putative placebo effects and/or the positive physiological response induced by sampling in injected patients. This strongly suggests that sham operated control groups are absolutely required to conclude on efficacy. The choice between heterogeneous BMMNC and BM-MSC may depend on the application, as demonstrated by the meta-analysis of large animal models of ischemic heart disease that reported significantly reduced efficacy of BMMNCs compared to BM-MSCs [40] whereas no difference was obvious for chronic kidney diseases [41]. Unexplained and recurrent discrepancies with autologous BMMNC in many clinical trials for heart diseases [47], as well as smaller suggested effects of BMMNC compared to BM-MSC for critical limb ischemia and foot ulcers, may participate in the progressive decline of the uses of heterogeneous fraction [48]. This could also explain the relative decline of investigations in the cardiac field. Furthermore, the increasing involvement of the pharmaceutical industry in cell therapy promotes the uses of cultured cells compared to the heterogeneous fraction most supported by the bio-devices industry [8, 49]. For MSCs, two-thirds of studies were run on an open-labeled design, revealing that this field has not yet reached maturity - but it should do so in the next few years given the number of phase 2 trials in various fields. Among MSC tissue sources, the increasing use of AT is consistent with the fact it is the richest adult source of MSCs, and is easy to sample by liposuction under local anesthesia

[50]. BM-MSCs benefit from their longer research history and the associated significant scientific hindsight [5, 51]. The power of our analysis, based on the MeSH ontology, gives the opportunity to reflect on pathophysiology when focusing on fields of application. This work thus highlighted the predominance of cultured cells for immune diseases or their consequences [37]. It is noteworthy that the uses of BM-MSCs for neurological disorders, and ASCs for graft-*vs*-host or rectal fistula were, as expected, far from and unrelated to the putative specific biological features due to the initial native environment. This emphasizes the possibility that the biological features of these cells have not been well-characterized and could reveal unexpected physiological features of these cells [51].

Allogeneic MSC trials have increased and their easy production, in large numbers from selected donors with no systemic pathology, may compensate for the decrease in MSC potentiality with age or pathological conditions, which may interfere with autologous grafts [14, 51].

The limitations of this study concerning the representativeness of this dataset should be considered. Other sources could have been consulted, such as the International Clinical Trials Registry Platform (ICTRP), which provides access to trials from several worldwide databases, including CTD (which accounts for about 60-80% [52, 53]). Unfortunately, ICTRP displays great disparities in data quality among the different registries, particularly for important elements such as primary outcomes and intervention details, which makes it difficult to conduct a systematic review [52]. CTD was chosen because it has more detailed and standardized exportable characteristics and registers a majority of trials. For instance, a search performed in ICTRP with the keywords “mesenchymal stem cells” indicated that at least 408 of 562 trials (73%) were registered in CTD. Furthermore, disease misclassification, although variable according to clinical specialties, is low [27] and some registered data have even been shown to better reflect reality than the final publications [54]. Another limitation was that registered trials only concern a part

of all existing trials [24]. It has been suggested that only 50% of clinical studies in Pubmed that involved administration of cells for regenerative medicine indicated any clinical trial identifier [53]. In fact, the final dataset is only the tip of the iceberg and the explosion of cell therapy outside the framework of academic research strongly concerns the scientific community. All around the world, private clinics already offer stem cell tourism, routinely injecting MSCs provided by some companies with non-validated or unproven procedures [55-58]. Since these clinics often use registries to gain legitimacy, an important implication of this work would be to carefully analyze final publications, especially from these companies, in order to assess the quality of and transparency of cell culture/procedures, cell controls, study design, reporting, placebo controlled efficacy and also transparency about cell culture/processing and ethical considerations [25, 59, 60]. Another limitation is semantic. The frequent confusion between expanded cells and heterogeneous fraction (particularly the term "stem" for CVD) does not facilitate analysis of this field. For instance, in NCT01788059, "injection [of] mesenchymal stem cell" was employed whereas the authors described the isolation of BMMNCs cells using Ficoll gradient. Similarly, the term adipose-derived stem cells has been employed by some authors to describe the injection of stromal vascular fraction (e.g. NCT02216630 or NCT01586715). Now, although almost 30% of studies have been terminated or completed, only 1% of the studies have posted results on CTD. For the future and for all cells and applications, the registration of final results will be required to fight against bias in analysis that results from the fact that negative outcomes are rarely published.

Conclusion

Stem cell research is challenged by two contradictory trends: diminished funding from public and private sponsors contrasted with increased scientific opportunities [61, 62]. Our work demonstrates the power of wide-reaching analysis, which could reveal unexpected facts and lead to the development of new concepts from already-available data. Such an approach should draw parallels between *in-vitro*, *in-vivo* and human data. Exchanges in both directions between preclinical and clinical research could optimize the parameters of clinical trials step by step. Such optimization would include the best sources of stem cells, the choice between heterogeneous or purified cells, and between allogeneic or autologous cell sources, taking the pathophysiology of diseases and the patients' characteristics into account.

References

1. Medvinsky A, Livesey FJ. On human development: lessons from stem cell systems. **Development**. 2015;142:17-20.
2. Collins FS. Exceptional opportunities in medical science: a view from the National Institutes of Health. **JAMA**. 2015;313:131-132.
3. Hauser SL. Hematopoietic stem cell transplantation for MS: extraordinary evidence still needed. **JAMA**. 2015;313:251-252.
4. Harousseau JL, Moreau P. Autologous hematopoietic stem-cell transplantation for multiple myeloma. **N Engl J Med**. 2009;360:2645-2654.
5. Friedenstein AJ, Chailakhjan RK, Lalykina KS. The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. **Cell Tissue Kinet**. 1970;3:393-403.

6. Murphy MB, Moncivais K, Caplan AI. Mesenchymal stem cells: environmentally responsive therapeutics for regenerative medicine. **Exp Mol Med**. 2013;45:e54.

7. Dominici M, Le Blanc K, Mueller I et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. **Cytotherapy**. 2006;8:315-317.

8. Breakdown of Mesenchymal Stem Cell Clinical Trials, by Phase, Geography, Sponsors, and More. Available at: http://www.bioinformant.com/global-breakdown-of-mesenchymal-stem-cell-clinical-trials-by-phase/.

9. Squillaro T, Peluso G, Galderisi U. Clinical Trials with Mesenchymal Stem Cells: An Update. **Cell Transplant**. 2015.

10. Aldhous P. A world of difference. **Nature**. 2001;414:838.

11. Lalu MM, McIntyre L, Pugliese C et al. Safety of cell therapy with mesenchymal stromal cells (SafeCell): a systematic review and meta-analysis of clinical trials. **PLoS One**. 2012;7:e47559.

12. Higgins J, Green S. Cochrane Handbook for Systematic Reviews of Interventions. **The Cochrane Collaboration**. 2009.

13. Vonk LA, de Windt TS, Slaper-Cortenbach IC et al. Autologous, allogeneic, induced pluripotent stem cell or a combination stem cell therapy? Where are we headed in cartilage repair and why: a concise review. **Stem Cell Res Ther**. 2015;6:94.

14. Minguell JJ, Allers C, Lasala GP. Mesenchymal stem cells and the treatment of conditions and diseases: the less glittering side of a conspicuous stem cell for basic research. **Stem Cells Dev**. 2013;22:193-203.

15. Weber GM, Mandl KD, Kohane IS. Finding the missing link for big biomedical data. **JAMA**. 2014;311:2479-2480.

16. Zaslavsky A, Perera C, Georgakopoulos D. Sensing as a Service and Big Data. http://arxivorg/ftp/arxiv/papers/1301/13010159pdf

17. Murdoch TB, Detsky AS. The inevitable application of big data to health care. **JAMA**. 2013;309:1351-1352.

18. Gough D, Thomas J, Oliver S. Clarifying differences between review designs and methods. **Syst Rev**. 2012;1:28.

19. Trounson A, Thakar RG, Lomax G et al. Clinical trials for stem cell therapies. **BMC Med**. 2011;9:52.

20. Grant MJ, Booth A. A typology of reviews: an analysis of 14 review types and associated methodologies. **Health Info Libr J**. 2009;26:91-108.

21. Yuan Y, Hunt RH. Systematic reviews: the good, the bad, and the ugly. **Am J Gastroenterol**. 2009;104:1086-1092.

22. Wong B. Points of view: Visualizing biological data. **Nat Meth**. 2012;9:1131-1131.

23. Fayyad U, Piatetsky-Shapiro G, P S. From Data Mining to Knowledge Discovery in Databases. **AI Magazine**. 1996;17:37-54.

24. Dickersin K, Rennie D. The evolution of trial registries and their use to assess the clinical trial enterprise. **JAMA**. 2012;307:1861-1864.

25. Sharma RR, Pollock K, Hubel A et al. Mesenchymal stem or stromal cells: a review of clinical applications and manufacturing practices. **Transfusion**. 2014;54:1418-1437.

26. DeAngelis CD, Drazen JM, Frizelle FA et al. Clinical trial registration: a statement from the International Committee of Medical Journal Editors. **JAMA**. 2004;292:1363-1364.

27. Tasneem A, Aberle L, Ananth H et al. The database for aggregate analysis of ClinicalTrials.gov (AACT) and subsequent regrouping by clinical specialty. **PLoS One**. 2012;7:e33677.

28. Sensebe L, Gadelorge M, Fleury-Cappellesso S. Production of mesenchymal stromal/stem cells according to good manufacturing practices: a review. **Stem Cell Res Ther**. 2013;4:66.

29. Califf RM, Zarin DA, Kramer JM et al. Characteristics of clinical trials registered in ClinicalTrials.gov, 2007-2010. **JAMA**. 2012;307:1838-1847.

30. Letunic I, Bork P. Interactive Tree Of Life (iTOL): an online tool for phylogenetic tree display and annotation. **Bioinformatics**. 2007;23:127-128.

31. Krzywinski M, Schein J, Birol I et al. Circos: an information aesthetic for comparative genomics. **Genome Res**. 2009;19:1639-1645.

32. Kuehn BM. Advances aim to ease stem cell concerns. **JAMA**. 2005;294:2557-2558.

33. Kuehn BM. Stem cells created from somatic cells. **JAMA**. 2005;294:1475-1476.

34. Aldhous P. Can they rebuild us? **Nature**. 2001;410:622-625.

35. A human stem cell project? **Nature**. 2002;418:1.

36. Thomson JA, Itskovitz-Eldor J, Shapiro SS et al. Embryonic stem cell lines derived from human blastocysts. **Science**. 1998;282:1145-1147.

37. Uccelli A, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. **Nat Rev Immunol**. 2008;8:726-736.

38. Ren G, Chen X, Dong F et al. Concise review: mesenchymal stem cells and translational medicine: emerging issues. **Stem Cells Transl Med**. 2012;1:51-58.

39. Ilic D. Trends in the stem cell and regenerative medicine industry. **Regen Med**. 2012;7:645-648.

40. van der Spoel TI, Jansen of Lorkeers SJ, Agostoni P et al. Human relevance of pre-clinical studies in stem cell therapy: systematic review and meta-analysis of large animal models of ischaemic heart disease. **Cardiovasc Res**. 2011;91:649-658.

41. Papazova DA, Oosterhuis NR, Gremmels H et al. Cell-based therapies for experimental chronic kidney disease: a systematic review and meta-analysis. **Dis Model Mech**. 2015;8:281-293.

42. Nisbet MC. The Competition for Worldviews: Values, Information, and Public Support for Stem Cell Research. **International Journal of Public Opinion Research**. 2005;17:90-112.

43. Pavo N, Charwat S, Nyolczas N et al. Cell therapy for human ischemic heart diseases: Critical review and summary of the clinical experiences. **J Mol Cell Cardiol**. 2014;75c:12-24.

44. Fisher SA, Brunskill SJ, Doree C et al. Stem cell therapy for chronic ischaemic heart disease and congestive heart failure. **Cochrane Database Syst Rev**. 2014;4:Cd007888.

45. Clifford DM, Fisher SA, Brunskill SJ et al. Stem cell treatment for acute myocardial infarction. **Cochrane Database Syst Rev**. 2012;2:Cd006536.

46. Jeong H, Yim HW, Cho Y et al. The effect of rigorous study design in the research of autologous bone marrow-derived mononuclear cell transfer in patients with acute myocardial infarction. **Stem Cell Res Ther**. 2013;4:82.

47. Francis DP, Mielewczik M, Zargaran D et al. Autologous bone marrow-derived stem cell therapy in heart disease: discrepancies and contradictions. **Int J Cardiol**. 2013;168:3381-3403.

48. Lu D, Chen B, Liang Z et al. Comparison of bone marrow mesenchymal stem cells with bone marrow-derived mononuclear cells for treatment of diabetic critical limb ischemia and foot ulcer: a double-blind, randomized, controlled trial. **Diabetes Res Clin Pract**. 2011;92:26-36.

49. TiGenix. TiGenix announces Cx601 meets primary endpoint in pivotal Phase III trial Brussels Stock Exchange:TIG. Available at: http://globenewswire.com/news-release/2015/08/23/762658/10146900/en/TiGenix-announces-Cx601-meets-primary-endpoint-in-pivotal-Phase-III-trial.html.

50. Bourin P, Sensebé L, Planat-Bénard V et al. Culture and Use of Mesenchymal Stromal Cells in Phase I and II Clinical Trials. **Stem Cells Int**. 2010;2010:503593.

51. Kern S, Eichler H, Stoeve J et al. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. **Stem Cells**. 2006;24:1294-1301.

52. Viergever RF, Karam G, Reis A et al. The quality of registration of clinical trials: still a problem. **PLoS One**. 2014;9:e84727.

53. Bersenev A. Cell therapy clinical trials – 2014 report. CellTrials blog.; January 22, 2015. Available: http://celltrials.info/2015/01/22/2014-report/.

54. Riveros C, Dechartres A, Perrodeau E et al. Timing and Completeness of Trial Results Posted at ClinicalTrials.gov and Published in Journals. **PLoS Med**. 2013;10:e1001566.

55. Cyranoski D. Stem cells in Texas: Cowboy culture. **Nature**. 2013;494:166-168.

56. Cyranoski D. Stem-cell therapy takes off in Texas. **Nature**. 2012;483:13-14.

57. Gunter KC, Caplan AL, Mason C et al. Cell therapy medical tourism: time for action. **Cytotherapy**. 2010;12:965-968.

58. Cyranoski D. Stem-cell therapy faces more scrutiny in China. **Nature**. 2009;459:146-147.

59. Isasi R. Registration of Stem Cell-Based Clinical Trials: A Scientific and Ethical Imperative. **World Stem Cell Report**. 2009.

60. Mendicino M, Bailey AM, Wonnacott K et al. MSC-based product characterization for clinical trials: an FDA perspective. **Cell Stem Cell**. 2014;14:141-145.

61. Moses H, 3rd, Matheson DH, Cairns-Smith S et al. The anatomy of medical research: US and international comparisons. **JAMA**. 2015;313:174-189.

62. Zerhouni EA. Research funding. NIH in the post-doubling era: realities and strategies. **Science**. 2006;314:1088-1090.

Acknowledgements

This study was supported by funding from the Midi-Pyrenees region, Paul Sabatier University and the research platform from Toulouse Dental Faculty. The authors thank Susan Becker for proofreading. We declare no conflicts of interest.

Figure 1. Temporal evolution of fields of application for regenerative medicine by stem cells.

Studies using hematopoietic stem cell transplant (with total body irradiation, myeloablative or non-myeloablative regimens, or genetically modified HSCs) were excluded from this figure. This chord diagram represents the proportion of studies dealing with each field of application (branch of the MeSH classification [Diseases]) and links them to their respective start dates.
The gray levels for the different years (on the left) and the color codes for the different fields of application (on the right) are shown beside their respective labels, listed in order of frequency (this order will be maintained hereafter). The outer ring of the figure contains the proportion of studies while the inner circle shows their absolute numbers. In each ring, the contribution of each field of application in each time category is coded by colored segments, and vice versa. These colored segments are also sorted by importance.
For instance, and reading the diagram from MeSH classification, in the field of cardiovascular regeneration, 70 trials started in 2006-2008 (indicated in the inner part of the circular diagram) and made up 36% of the fields in 2006-2008 (indicated on the left of the outer part of the circular diagram). Conversely, 25% of cardiovascular diseases trials were started in 2006-2008 (indicated on the right of the outer part of the circular diagram).

Figure 2. Uses of the different types of stem cells in regenerative medicine

Studies using hematopoietic stem cell transplant (with total body irradiation, myeloablative or non-myeloablative regimens, or genetically modified HSCs) were excluded from this figure. This chord diagram represents the proportion of studies dealing with each field of application, linked to their respective uses of stem cells. The color codes for the different fields of application (on the left) and the color codes for the different types of stem cells (on the right) are shown beside their respective labels.

Abbreviations for cells: muco-MSC: mucosal Mesenchymal Stem Cell; men-MSC: menstrual Mesenchymal Stem Cell; dent-MSC: Mesenchymal Stem Cell from dental tissues; MSC: Mesenchymal Stem Cell (probably BMSCs); ASC: Adipose-derived Stromal/Stem Cells; UC-MSC: Umbilical-Cord or Umbilical-Cord Blood Mesenchymal Stem Cell or Wharton Jelly MSC or placental MSCs; BM-MSC: Bone Marrow Mesenchymal Stem Cell.
mens-BSC: menstrual Blood Stem Cell; UCBSC: Umbilical Cord Blood Stem Cell; SVF: Stromal Vascular Fraction; BMMNC: Bone Marrow MonoNuclear Cell; PBSC: Peripheral Blood Stem Cell; EPC: Endothelial or Angiogenic Precursor Cell; CD133: CD133+; CD34: CD34+ cells.
MPC: Mesenchymal Precursor Cell; MSC-P: MSC-derived progenitors and cells (osteoprogenitor, MSC-derived cardiopoietic cell, MSC-derived neuroprogenitor, MSC-derived osteoprogenitor, MSC-derived hepatic cell, MSC-derived endometrium); CSC: Cardiosphere-Derived Cells, Cardiac Stem Cells; NSC: Neural Stem Cell; CLSC: Corneal Limbal Stem Cell or Retinal progenitor cell; mus-SC: Muscular Stem Cell; ESC-P: embryonic stem cell-derived cells (ESC-RPE: Embryonic Stem Cell Retinal Pigment Epithelial and other progenitors); skin-SC: Skin Stem Cell; OlG-P: Oligodendrocyte or glial progenitor; HSC-Ins: HSC-derived cell producing insulin.

Figure 3. Temporal evolution of fields of application for regenerative medicine by MSCs.

This chord diagram represents the proportion of studies dealing with each field of application, linked to the corresponding start year of trials. This formally illustrates the burst of cell therapy by MSCs between 1999 and today. The following MSCs were merged: muco-MSC, men-MSC, dent-MSC, ASC, UC-MSC and BM-MSC. For more details about abbreviations for cells, please see the legend of Figure 2. The gray levels for the different years (on the left) and the color codes for the different fields of application (on the right) are shown beside their respective labels. For each time period, the total number of studies, and the relative number of studies for cardiovascular, nervous system and immune system diseases, are represented by a histogram in the upper part of the figure.

Figure 4. State of progress of MSCs uses in regenerative medicine

This chord diagram exposes the relationships between application fields and study phases for MSCs, which reveals the enthusiasm for such therapy and the fact that we will soon know the efficacy of these therapies in a large number of fields of application. The following MSCs were merged: muco-MSC, men-MSC, dent-MSC, ASC, UC-MSC and BM-MSC. For more details about abbreviations for cells, please see the legend of Figure 2. The color codes for the different study phases (on the left) and the color codes for the different fields of application (on the right) are shown beside their respective labels. The ratio of the number of Phase 1, 1/2 trials to the number of Phase 2, 2/3 and 3 trials for cardiovascular, nervous system, immune system, digestive system, respiratory tract and endocrine system diseases, are represented by a histogram in the upper part of the figure.

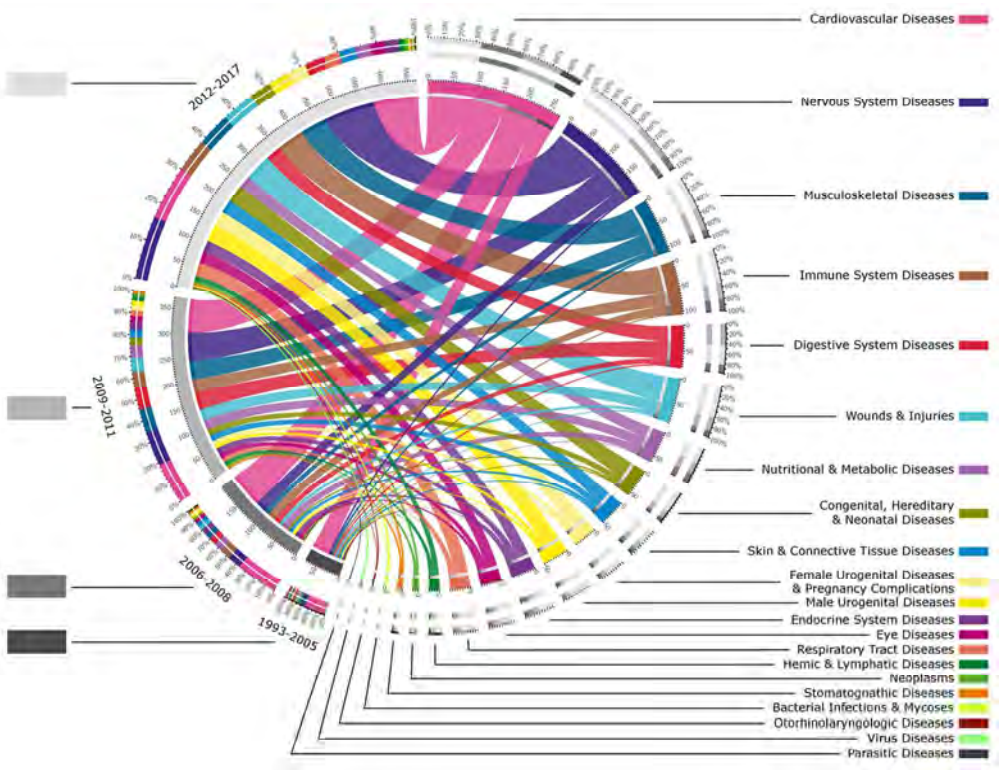


Figure 1. Temporal evolution of fields of application for regenerative medicine by stem cells.\r\n\r\nStudies using hematopoietic stem cell transplant (with total body irradiation, myeloablative or non-myeloablative regimens, or genetically modified HSCs) were excluded from this figure. This chord diagram represents the proportion of studies dealing with each field of application (branch of the MeSH classification [Diseases]) and links them to their respective start dates.
The gray levels for the different years (on the left) and the color codes for the different fields of application (on the right) are shown beside their respective labels, listed in order of frequency (this order will be maintained hereafter). The outer ring of the figure contains the proportion of studies while the inner circle shows their absolute numbers. In each ring, the contribution of each field of application in each time category is coded by colored segments, and vice versa. These colored segments are also sorted by importance.
For instance, and reading the diagram from MeSH classification, in the field of cardiovascular regeneration, 70 trials started in 2006-2008 (indicated in the inner part of the circular diagram) and made up 36% of the fields in 2006-2008 (indicated on the left of the outer part of the circular diagram). Conversely, 25% of cardiovascular diseases trials were started in 2006-2008 (indicated on the right of the outer part of the circular diagram).
110x84mm (300 x 300 DPI)

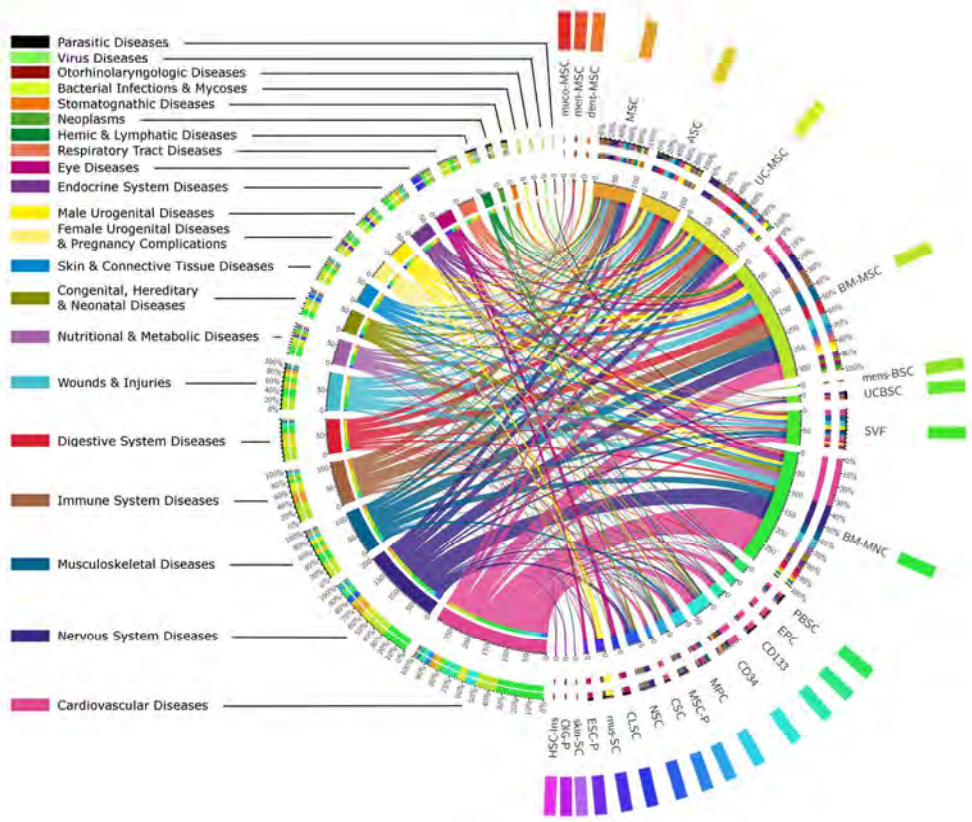


Figure 2. Uses of the different types of stem cells in regenerative medicine.

Studies using hematopoietic stem cell transplant (with total body irradiation, myeloablative or non-myeloablative regimens, or genetically modified HSCs) were excluded from this figure. This chord diagram represents the proportion of studies dealing with each field of application, linked to their respective uses of stem cells. The color codes for the different fields of application (on the left) and the color codes for the different types of stem cells (on the right) are shown beside their respective labels.

Abbreviations for cells: muco-MSC: mucosal Mesenchymal Stem Cell; men-MSC: menstrual Mesenchymal Stem Cell; dent-MSC: Mesenchymal Stem Cell from dental tissues; MSC: Mesenchymal Stem Cell (probably BMSCs); ASC: Adipose-derived Stromal/Stem Cells; UC-MSC: Umbilical-Cord or Umbilical-Cord Blood Mesenchymal Stem Cell or Wharton Jelly MSC or placental MSCs; BM-MSC: Bone Marrow Mesenchymal Stem Cell.
mens-BSC: menstrual Blood Stem Cell; UCBSC: Umbilical Cord Blood Stem Cell; SVF: Stromal Vascular Fraction; BMMNC: Bone Marrow MonoNuclear Cell; PBSC: Peripheral Blood Stem Cell; EPC: Endothelial or Angiogenic Precursor Cell; CD133: CD133+; CD34: CD34+ cells.
MPC: Mesenchymal Precursor Cell; MSC-P: MSC-derived progenitors and cells (osteoprogenitor, MSC-derived cardiopoietic cell, MSC-derived neuroprogenitor, MSC-derived osteoprogenitor, MSC-derived hepatic cell, MSC-derived endometrium); CSC: Cardiosphere-Derived Cells, Cardiac Stem Cells; NSC: Neural Stem Cell; CLSC: Corneal Limbal Stem Cell or Retinal progenitor cell; mus-SC: Muscular Stem Cell; ESC-P: embryonic stem cell-derived cells (ESC-RPE: Embryonic Stem Cell Retinal Pigment Epithelial and other progenitors); skin-SC: Skin Stem Cell; OlG-P: Oligodendrocyte or glial progenitor; HSC-Ins: HSC-derived cell producing insulin.
118x102mm (300 x 300 DPI)

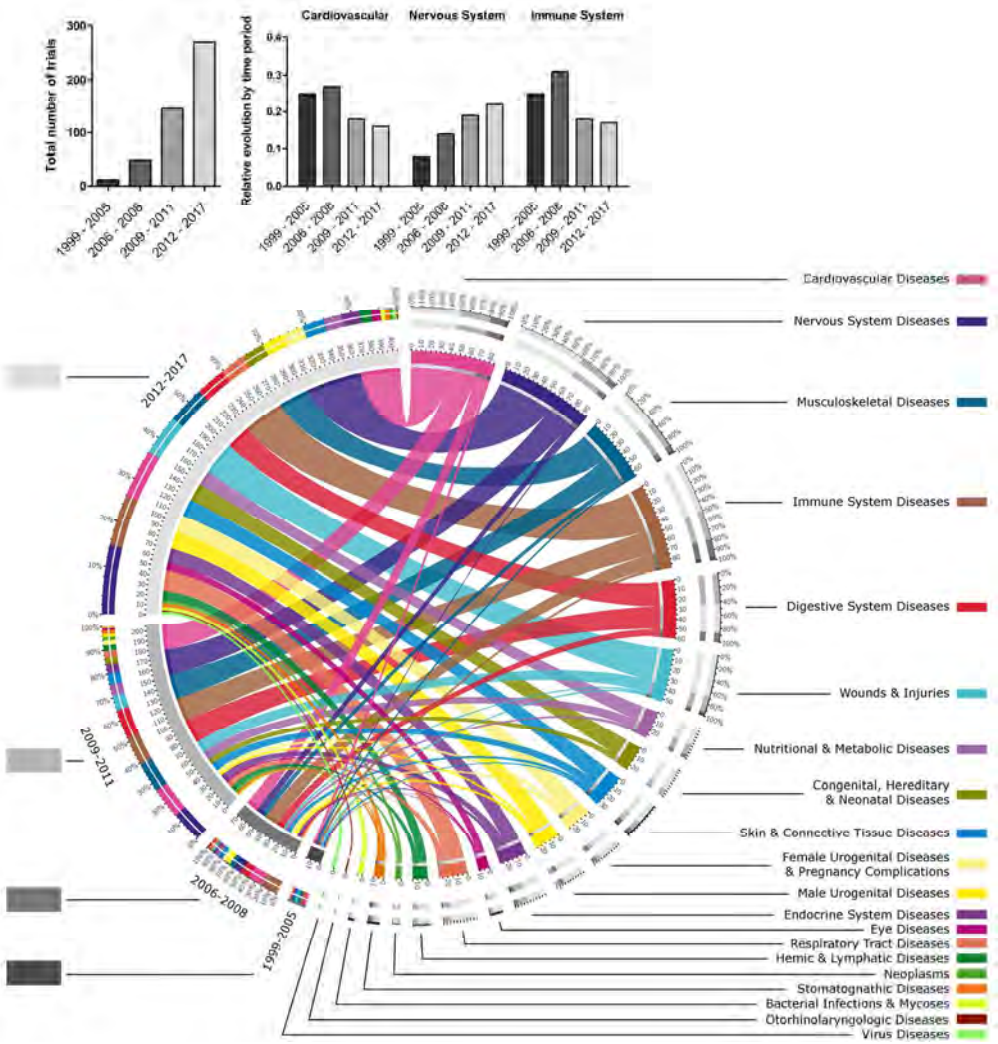


Figure 3. Temporal evolution of fields of application for regenerative medicine by MSCs.

This chord diagram represents the proportion of studies dealing with each field of application, linked to the corresponding start year of trials. This formally illustrates the burst of cell therapy by MSCs between 1999 and today. The following MSCs were merged: muco-MSC, men-MSC, dent-MSC, ASC, UC-MSC and BM-MSC. For more details about abbreviations for cells, please see the legend of Figure 2. The gray levels for the different years (on the left) and the color codes for the different fields of application (on the right) are shown beside their respective labels. For each time period, the total number of studies, and the relative number of studies for cardiovascular, nervous system and immune system diseases, are represented by a histogram in the upper part of the figure.
148x154mm (300 x 300 DPI)

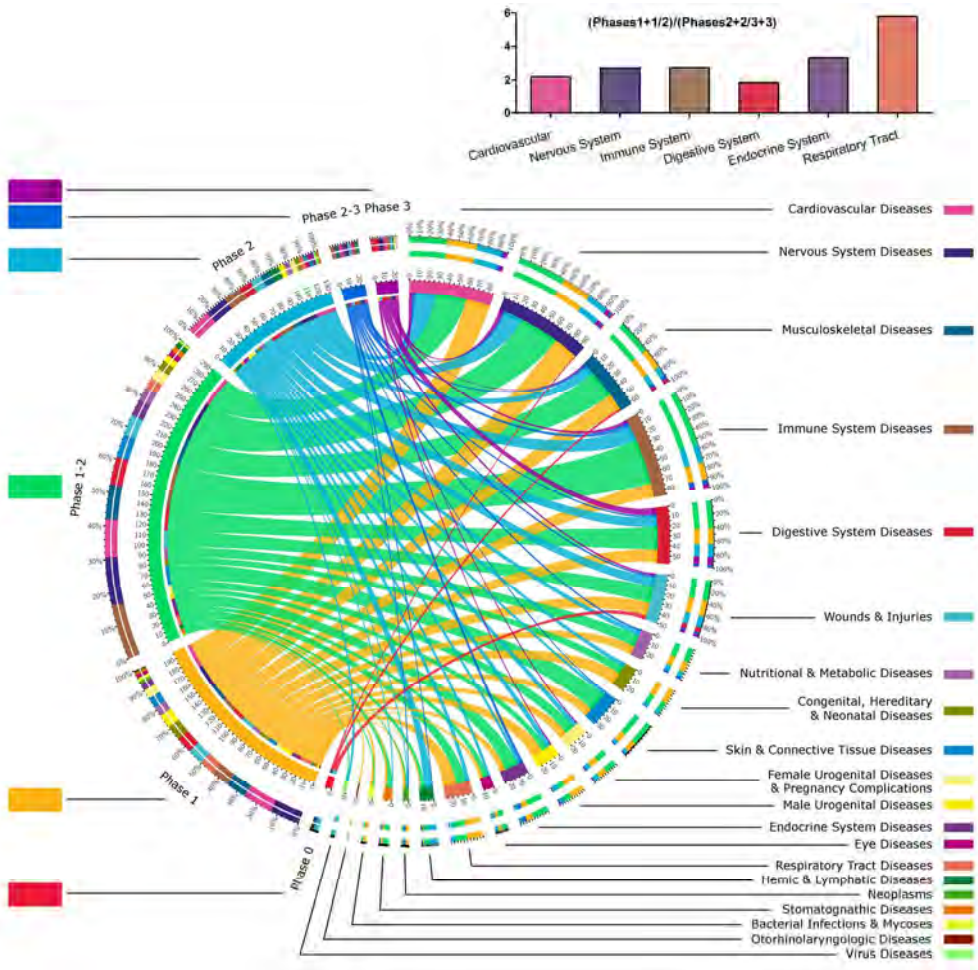


Figure 4. State of progress of MSCs uses in regenerative medicine.

This chord diagram exposes the relationships between application fields and study phases for MSCs, which reveals the enthusiasm for such therapy and the fact that we will soon know the efficacy of these therapies in a large number of fields of application. The following MSCs were merged: muco-MSC, men-MSC, dent-MSC, ASC, UC-MSC and BM-MSC. For more details about abbreviations for cells, please see the legend of Figure 2. The color codes for the different study phases (on the left) and the color codes for the different fields of application (on the right) are shown beside their respective labels. The ratio of the number of Phase 1, 1/2 trials to the number of Phase 2, 2/3 and 3 trials for cardiovascular, nervous system, immune system, digestive system, respiratory tract and endocrine system diseases, are represented by a histogram in the upper part of the figure.
139x135mm (300 x 300 DPI)

	n	R_S	R_{BM}	R_{AT}	$R_{BM/AT}$
Total	***362***	***0.78***	***0.73***	***0.95***	***3.40***
Musculoskeletal Diseases	**45**	0.61	0.50	1.00	2.56
Digestive System Diseases	**27**	0.30	0.27	0.36	2.00
Respiratory Tract Diseases	**12**	0.58	0.38	1.67	3.14
Nervous System Diseases	**78**	0.88	0.97	0.50	5.48
Eye Diseases	**11**	1.00	0.89	2.00	5.67
Male Urogenital Diseases	**20**	0.70	0.43	1.33	1.43
Female Urogenital Diseases & Pregnancy Complications	**16**	0.38	0.31	0.50	1.42
Cardiovascular Diseases	**125**	1.72	1.82	1.18	6.70
Skin & Connective Tissue Diseases	**16**	0.52	0.32	1.50	2.40
Nutritional & Metabolic Diseases	**26**	1.14	1.00	2.50	5.29
Endocrine System Diseases	**20**	0.89	0.88	1.00	4.67
Immune System Diseases	**18**	0.18	0.13	0.67	7.00
Wounds & Injuries	**44**	0.69	0.61	0.85	2.22
Before 2006	**33**	2.67	2.91	-	42.0
2006-2008	**64**	1.21	1.24	1.00	7.75
2009-2011	**100**	0.77	0.83	0.52	4.66
After 2011	**162**	0.58	0.39	1.22	2.11
Phase 0 and 1	**92**	0.60	0.64	0.46	3.58
Phase 1-2 and 2	**201**	0.76	0.69	1.02	3.24
Phase 2-3 and 3	**32**	0.93	1.04	0.25	10.4

Table 1. Comparison between culture expanded MSCs and the respective heterogeneous fractions, from bone marrow or adipose tissue

The table presents some statistics computed from these data, for a better understanding of the evolution of the use of bone marrow cells compared to adipose tissue cells and the use of heterogeneous fraction compared to expanded cells.
R_S is the ratio (SVF+BM-MNC)/ (ASC+BMSC+MSC),
R_{AT} is the ratio SVF/ASC,
R_{BM} is the ratio BM-MNC/ (BMSC+MSC),
$R_{BM/AT}$ is the ratio (BM-MNC+BMSC+MSC)/(SVF+ASC).

SUPPLEMENTARY INFORMATION

Supplementary Figure 1.
Program flow of the Perl-based script.

Supplementary Figure 2.
Annotated figure about uses of the different types of stem cells in regenerative medicine.

Supplementary Figure 3.
PRISMA flow diagram of detailed screening process.

Supplementary Figure 4.
Comparison of expanded MSCs and the respective heterogeneous fraction, from bone marrow or adipose tissue.

Supplementary Figure 5.
Screenshot of the website http://multireview.perso.sfr.fr/.

Supplementary Figure 6.
Use of ASCs in regenerative medicine.

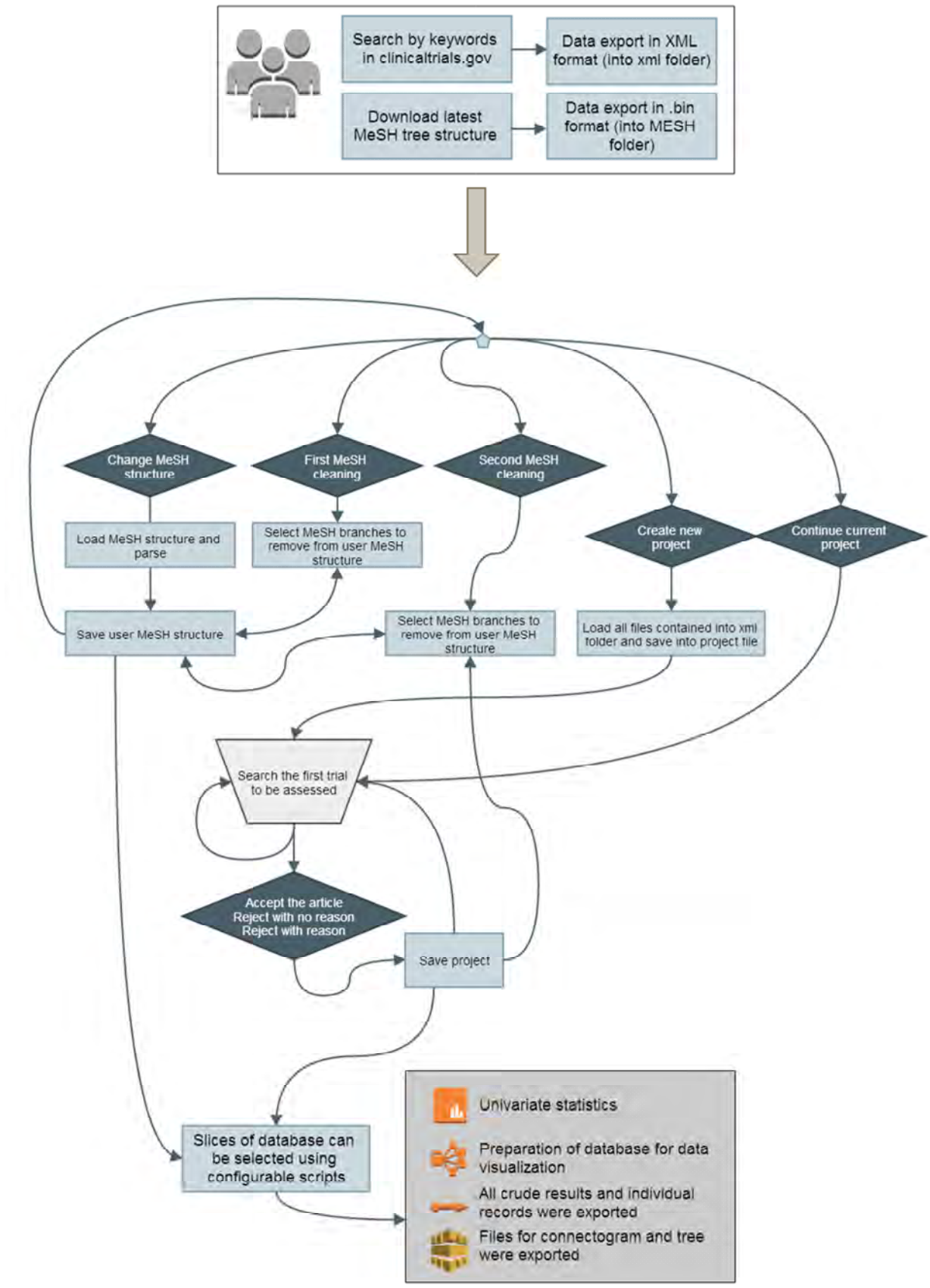
Supplementary Table 1.
List of packages employed in Perl script.

Supplementary Table 2.
Characteristics of trials.

Supplementary Table 3.

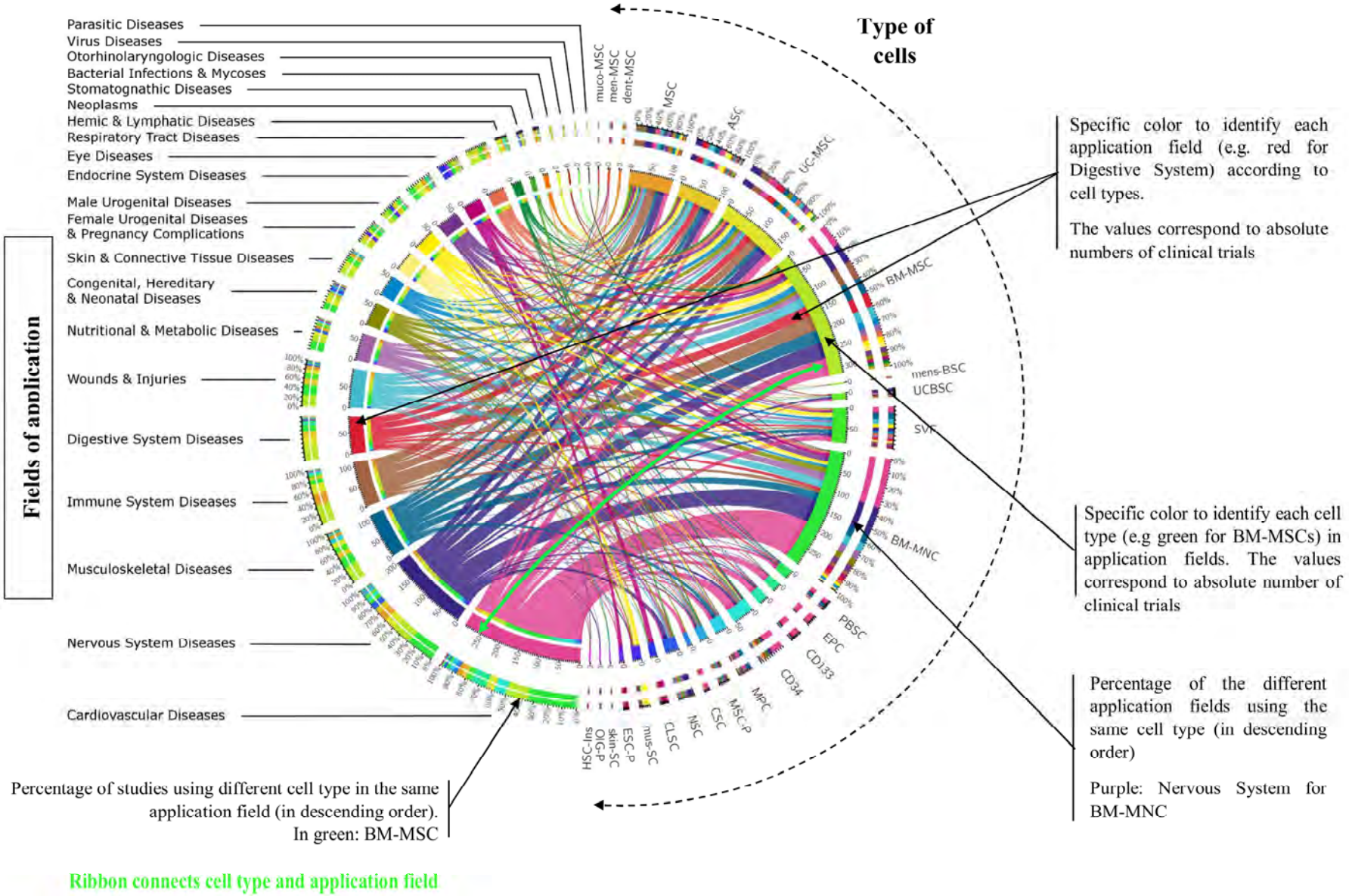
List of cellular specialties identified during screening, with the description of the type of cells (cellular source, allogeneic or autologous), and the number of trials.

Supplementary File 1.
Refinement of the resulting MeSH terms before phylogenetic-like tree visualization.



Supplementary Figure 1.
Program flow of the Perl-based script

Data aggregation was performed using a Perl script we developed, to minimize errors during the screening process and to computerize data for further analyses.



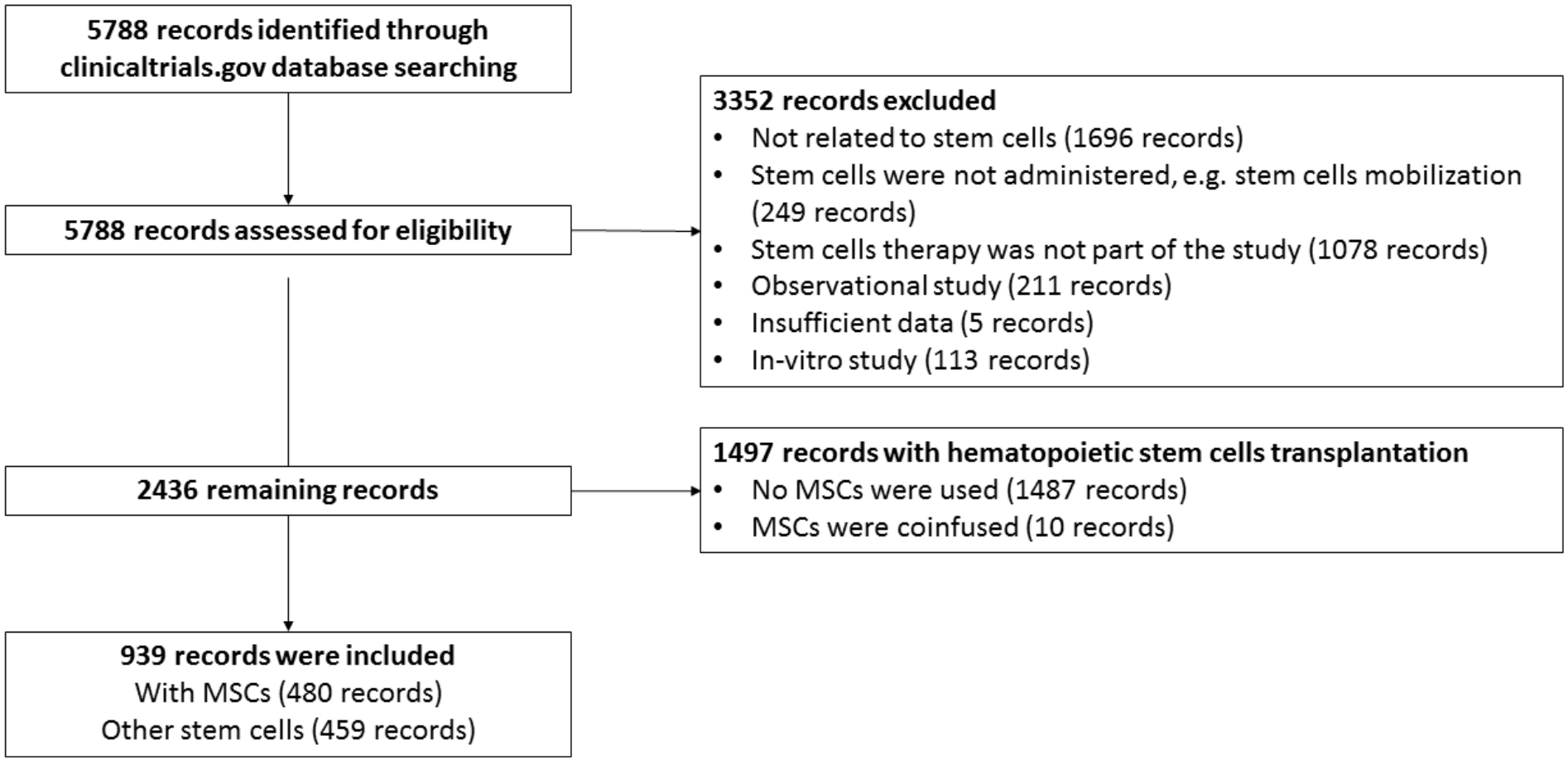
Supplementary Figure 2.
Annotated figure about uses of the different types of stem cells in regenerative medicine

Studies using hematopoietic stem cell transplant (with total body irradiation, myeloablative or non-myeloablative regimens, or genetically modified HSCs) were excluded from this figure. This chord diagram represents the proportion of studies dealing with each field of application, linked to their respective uses of stem cells.

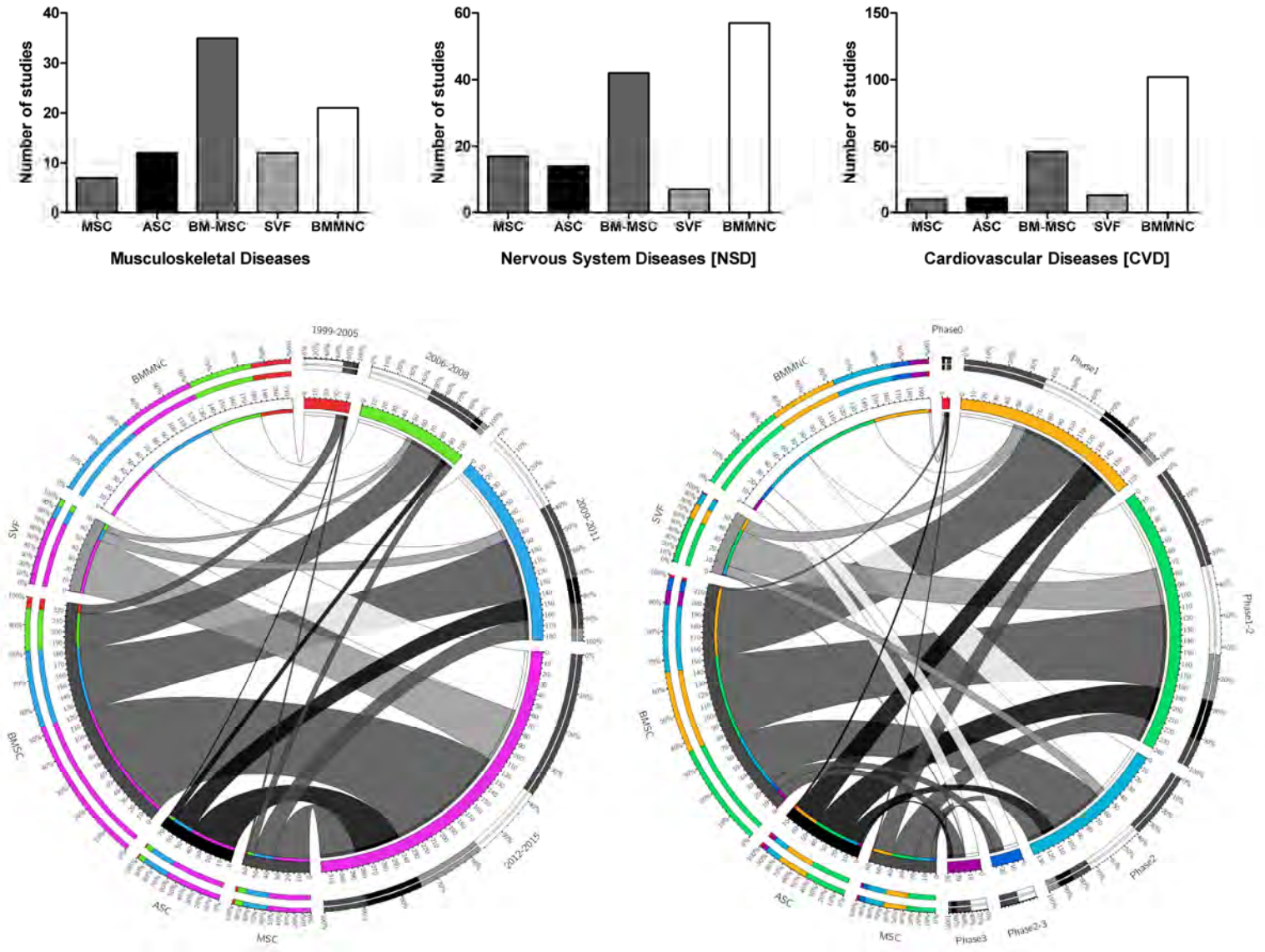
Abbreviations for cells: muco-MSC: mucosal Mesenchymal Stem Cell; men-MSC: menstrual Mesenchymal Stem Cell; dent-MSC: Mesenchymal Stem Cell from dental tissues; MSC: Mesenchymal Stem Cell (probably BMSCs); ASC: Adipose-derived Stromal/Stem Cells; UC-MSC: Umbilical-Cord or Umbilical-Cord Blood Mesenchymal Stem Cell or Wharton Jelly MSC or placental MSCs; BM-MSC: Bone Marrow Mesenchymal Stem Cell.

mens-BSC: menstrual Blood Stem Cell; UCBSC: Umbilical Cord Blood Stem Cell; SVF: Stromal Vascular Fraction; BMMNC: Bone Marrow MonoNuclear Cell; PBSC: Peripheral Blood Stem Cell; EPC: Endothelial or Angiogenic Precursor Cell; CD133: CD133+; CD34: CD34+ cells.

MPC: Mesenchymal Precursor Cell; MSC-P: MSC-derived progenitors and cells (osteoprogenitor, MSC-derived cardiopoietic cell, MSC-derived neuroprogenitor, MSC-derived osteoprogenitor, MSC-derived hepatic cell, MSC-derived endometrium); CSC: Cardiosphere-Derived Cells, Cardiac Stem Cells; NSC: Neural Stem Cell; CLSC: Corneal Limbal Stem Cell or Retinal progenitor cell; mus-SC: Muscular Stem Cell; ESC-P: embryonic stem cell-derived cells (ESC-RPE: Embryonic Stem Cell Retinal Pigment Epithelial and other progenitors); skin-SC: Skin Stem Cell; OlG-P: Oligodendrocyte or glial progenitor; HSC-Ins: HSC-derived cell producing insulin.



Supplementary Figure 3.
PRISMA flow diagram of detailed screening process



Supplementary Figure 4.

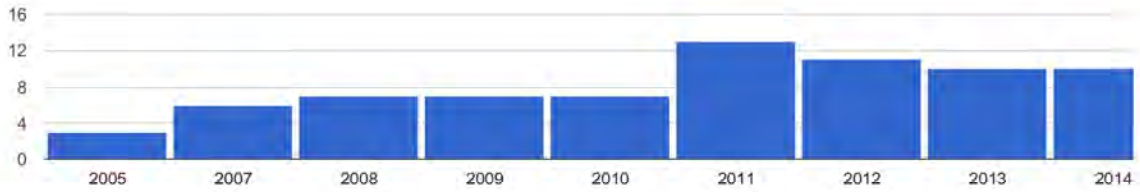
Comparison of expanded MSCs and the respective heterogeneous fraction, from bone marrow or adipose tissue

The histograms of the upper part of figure show the number of trials using MSCs, ASCs, BM-MSCs, SVF or BMMNCs for musculoskeletal diseases, NSD and CVD. The lower part of the figure represents the proportion of studies dealing with each type of cells, with the start years of trials and study phases.

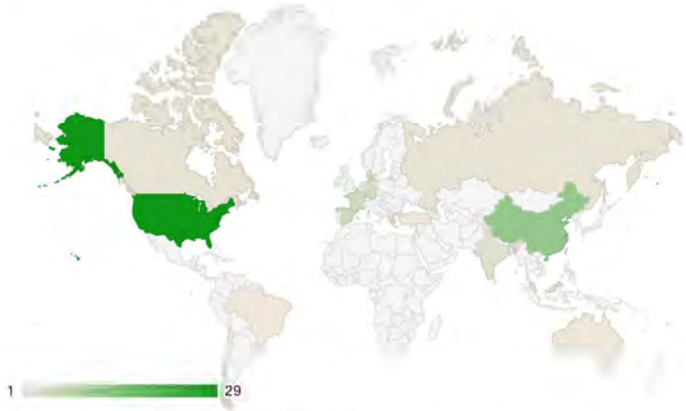
Back to the list

Cardiovascular Diseases

Histogram of years of publication of studies



Country of origin



List of trials for this MeSH descriptor

NCT02323477; NCT02283879; NCT01714167; NCT01716481; NCT01351610; NCT01824069; NCT01219452; NCT01720888; NCT00518401; NCT00260338; NCT02315027; NCT01211028; NCT01449032; NCT00644410; NCT00548613; NCT01739777; NCT00587990; NCT02287831; NCT01468064; NCT01795950; NCT02378974; NCT01745744; NCT01287936; NCT00550498; NCT01091701; NCT00911365; NCT01297413; NCT01770613; NCT01922908; NCT01753440; NCT00790764; NCT01840540; NCT01759212; NCT02145897; NCT00721006; NCT01087996; NCT01302015; NCT00114452; NCT00643981; NCT02304588; NCT01394432; NCT01076920; NCT01065337; NCT01484574; NCT00875654; NCT00919958; NCT02266394; NCT01678534; NCT00951210; NCT01558908; NCT01957826; NCT01291329; NCT00883727; NCT01652209; NCT00955669; NCT02368587; NCT01389453; NCT00908856; NCT02123706; NCT02013674; NCT02336646; NCT01670981; NCT01310114; NCT00768066; NCT01913886; NCT01461720; NCT01557543; NCT01298830; NCT02394886; NCT02398604; NCT02408432; NCT02439541; NCT02448641; NCT02460770; NCT02462330; NCT00877903; NCT01392625; NCT01392105; NCT01436123; NCT01216865; NCT01257776; NCT01686139; NCT02274428; NCT01849887; NCT01962233; NCT01946048;

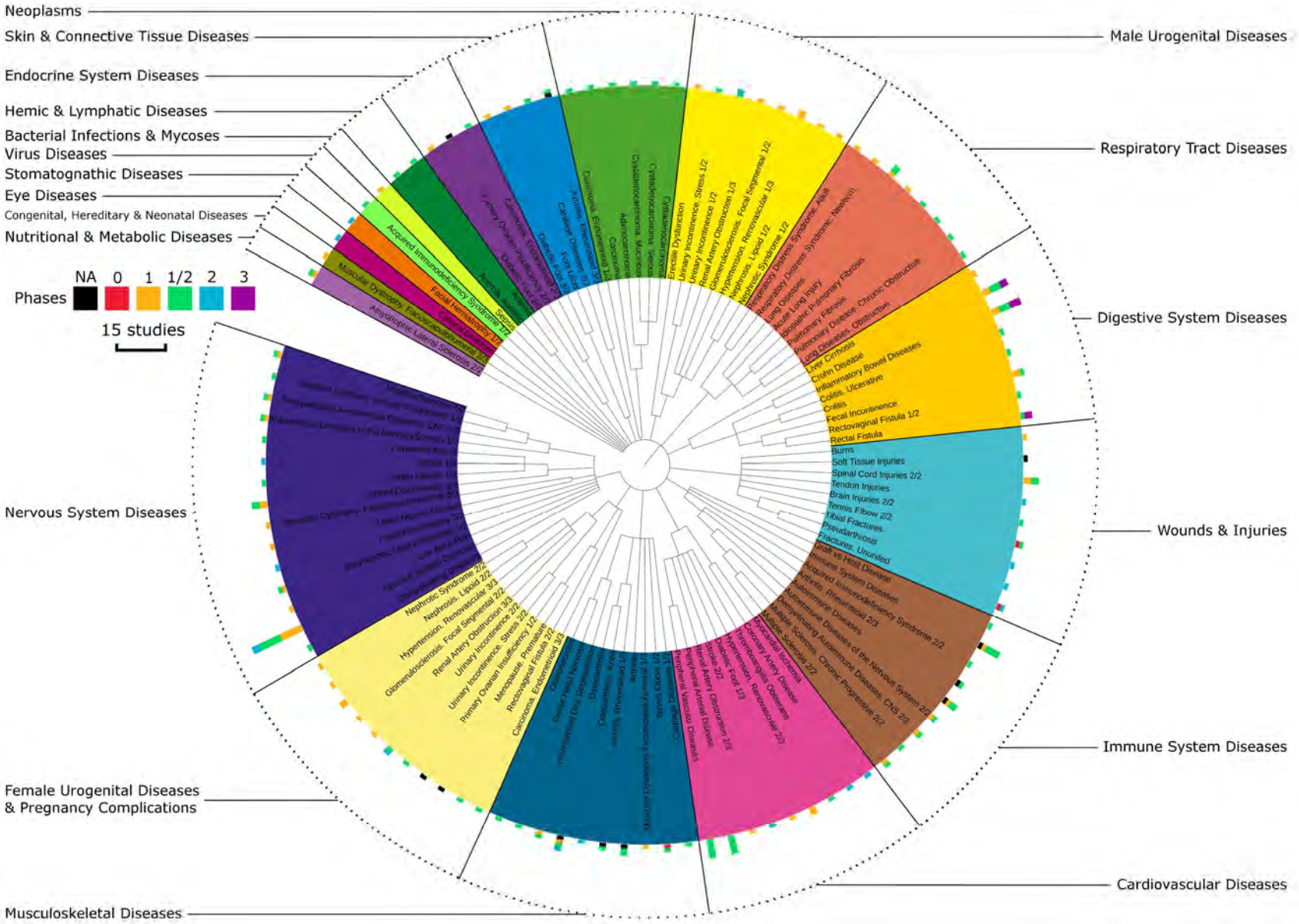
Study characteristics

	All studies	Percentage
State of the study	86	
Active, not recruiting	11	12.8%
Completed	22	25.6%
Not yet recruiting	17	19.8%
Recruiting	30	34.9%
Suspended	1	1.2%
Terminated	4	4.7%
Withdrawn	1	1.2%
Study size	30 [18 ; 59]	
Median [Q1 ; Q3]	N = 85	
Gender	86	
Both	85	98.8%
Male	1	1.2%
Study type	86	

Interventional	86	100.0%
Randomization	67	
Non-Randomized	14	20.9%
Randomized	53	79.1%
Intervention	86	
Crossover Assignment	1	1.2%
Factorial Assignment	3	3.5%
Parallel Assignment	51	59.3%
Single Group Assignment	31	36.0%
Blinding	86	
Double Blind (Subject	28	32.6%
Open Label	52	60.5%
Single Blind (Subject)	6	7.0%
Main sponsor	86	
Industry	31	36.0%
NIH	3	3.5%
Other	52	60.5%
Study phase	86	
Phase 1	26	30.2%
Phase 1/Phase 2	33	38.4%
Phase 2	21	24.4%
Phase 2/Phase 3	3	3.5%
Phase 3	3	3.5%
Study has DMC	78	
No	18	23.1%
Yes	60	76.9%
Donor	86	
Autologous	45	52.3%
Allogeneic	43	50.0%
Employed stem cells	86	
Umbilical Cord Mesenchymal Stem Cells	14	16.3%
Placental Mesenchymal Stem Cells	4	4.7%
Bone Marrow Mesenchymal Stem Cells	46	53.5%
Adipose derived Mesenchymal Stem Cells	11	12.8%
Mesenchymal Stem Cells	10	11.6%
Menstrual Mesenchymal Stem Cells	1	1.2%
Endothelial Precursor Cells	6	7.0%
Genetic or viral modification of cells	2	2.3%
Stromal Vascular Fraction	1	1.2%
Bone Marrow Mononuclear Cells	5	5.8%
Taking medication concomitantly	1	
Immunophilins	1	100.0%

Supplementary Figure 5.
Screenshot of the website http://multireview.perso.sfr.fr/

The website http://multireview.perso.sfr.fr/ provides detailed results about studies (using stem cells, MSCs or ASCs) included for each MeSH keyword. This screenshot is an example of the CVD branch (C14 or Cardiovascular Diseases) for MSC therapy. It presents the temporal evolution of the trials, their geographical mapping, the list of included trials and all trial characteristics (state, size, gender, type, randomization, design, blinding, sponsoring, phases, donor, stem cells employed and medications taken concomitantly).



Supplementary Figure 6.
Use of ASCs in regenerative medicine

Phylogenetic-like tree of included studies using ASCs according to MeSH descriptors. All retrieved keywords from trials were considered to be terminal taxa and are represented around a phylogenetic-like tree. As MeSH is redundant, desired duplicates are identified to indicate that there are several occurrences (Supplementary File 1).

Ancestors of the tree are represented using MeSH sub-levels of these taxa, placed in the center of circle. The number of studies for each clinical phase (Phase 0: red, Phase 1: orange, Phase 1-2: green, Phase 2: pale blue, Phase 2-3: deep blue, Phase 3: purple, and not applicable: black) is illustrated in histograms at the extremity of each end branch of the tree. Bar heights refer to the scale provided.

The key message is both diversity in fields of application and diversity of diseases within some fields. For example, in musculoskeletal diseases, MeSH structure showed the use of ASCs in osteonecrosis, arthritis, muscular dystrophies, cartilage diseases and tennis elbow, in Phases 1 to Phase 2. A great diversity was also observed for the 14 nervous system diseases studies, fragmented into sub-subjects (e.g. spinal cord injuries, multiple sclerosis, brain injuries).

For digestive diseases, two major themes emerged, revealed by the MeSH structure: one theme for liver cirrhosis and one theme for the digestive system. We found Phase 3 studies only for the treatment of digestive system fistula and inflammatory bowel diseases.

Name of package	Description provided by CPAN	Version
Dancer	Lightweight yet powerful web application framework	1.3110
Storable	Persistent data structure mechanism	2.45
Data-Dumper	Convert data structure into perl code	2.136
XML-Simple	API to maintain XML (esp config files)	2.20
URI -Escape	General URI escaping/unescaping functions	3.31
LWP-UserAgent	Web user agent class	6.04
Spreadsheet -WriteExcel	Write to a cross-platform Excel binary file	2.39
Statistics-Descriptive	Descriptive statistical methods	3.0605

Supplementary Table 1.
List of packages employed in Perl script.

The script was developed and run using Strawberry Perl 5.16.3.1-64 bits on Windows 7 operating system (Professional edition 64 bits) with 6Go RAM memory.

	Studies without hematopoietic stem cell transplantation				
	All studies (n = 939)	**Studies using MSCs including ASCs (n = 480, 51.1% total)**	**Studies using MSCs excluding ASCs (n = 403, 42.9% total, 84.0% MSCs)**	**Studies using ASCs (n = 77, 8.2% total, 16.0% MSCs)**	**p**
State of the study	**939**	**480**	**403**	**77**	
Not yet recruiting	219 (23.3%)	118 (24.6%)	98 (24.4%)	20 (26.0%)	0.76
Recruiting	421 (44.8%)	230 (47.9%)	196 (48.6%)	34 (44.2%)	0.47
Enrolling by invitation	27 (2.9%)	13 (2.7%)	12 (3.0%)	1 (1.3%)	0.41
Suspended	10 (1.1%)	3 (0.6%)	3 (0.7%)	0 (0.0%)	0.45
Withdrawn	11 (1.2%)	3 (0.6%)	3 (0.7%)	0 (0.0%)	0.45
Completed	220 (23.4%)	100 (20.9%)	80 (19.9%)	20 (26.0%)	0.23
Terminated	28 (3.0%)	11 (2.3%)	9 (2.2%)	2 (2.6%)	0.84
No longer available	3 (0.3%)	2 (0.4%)	2 (0.5%)	0 (0.0%)	0.54
Study size (median [Q1 ; Q3])	25 [12 ; 55] N = 919	25 [10 ; 50] N = 477	27 [12 ; 50] N = 403	19 [10 ; 40] N = 76	0.02 *
Gender	**939**	**480**	**403**	**77**	
Female only	26 (2.8%)	12 (2.5%)	9 (2.2%)	3 (3.9%)	0.39
Male only	28 (3.0%)	14 (2.9%)	10 (2.5%)	4 (5.2%)	0.19
Both	885 (94.2%)	454 (94.6%)	384 (95.3%)	70 (90.9%)	0.12
Clinical trial site	**896**	**460**	**386**	**74**	
Eastern Asia	203 (22.7%)	160 (34.8%)	133 (34.5%)	27 (36.5%)	0.74
Northern Asia	16 (1.8%)	6 (1.3%)	6 (1.6%)	0 (0.0%)	0.28
South Central Asia	105 (11.7%)	39 (8.5%)	34 (8.8%)	5 (6.8%)	0.56
South East Asia	18 (2.0%)	11 (2.4%)	9 (2.3%)	2 (2.8%)	0.84
Western Asia and Middle East	22 (2.5%)	13 (2.8%)	12 (3.1%)	1 (1.4%)	0.40
Central and South America	63 (7.0%)	19 (4.1%)	16 (4.1%)	3 (4.1%)	0.97
North America	222 (24.8%)	94 (20.4%)	87 (22.5%)	7 (9.5%)	0.01 **
Europe	290 (32.4%)	131 (28.5%)	90 (23.3%)	41 (55.4%)	< 0.001 ***
Africa	15 (1.7%)	9 (2.0%)	9 (2.3%)	0 (0.0%)	0.18
Oceania	15 (1.7%)	12 (2.6%)	12 (3.1%)	0 (0.0%)	0.12
Randomization	**930**	**477**	**401**	**76**	
Single arm study	369 (39.7%)	180 (37.7%)	150 (37.4%)	30 (39.5%)	0.73
Randomized	401 (43.1%)	213 (44.7%)	176 (43.9%)	37 (48.7%)	0.44
Non-randomized	160 (17.2%)	84 (17.6%)	75 (18.7%)	9 (11.8%)	0.15
Blinding	**930**	**477**	**401**	**76**	
Open label	652 (70.1%)	346 (72.5%)	290 (72.3%)	56 (73.7%)	0.55
Single blind	84 (9.0%)	33 (6.9%)	26 (6.5%)	7 (9.2%)	0.35
Double blind	194 (20.9%)	98 (20.5%)	85 (21.2%)	13 (17.1%)	0.47
Donor	**918**	**464**	**388**	**76**	
Allogeneic	316 (34.4%)	248 (53.4%)	224 (57.7%)	24 (31.6%)	< 0.001 ***
Autologous	604 (65.8%)	218 (47.0%)	167 (43.0%)	52 (68.4%)	
Main sponsor	**939**	**480**	**403**	**76**	

NIH	14 (1.5%)	8 (1.7%)	8 (2.0%)	0 (0.0%)	0.22
Industry	278 (29.6%)	146 (30.4%)	114 (28.3%)	32 (41.6%)	0.02 *
Other	647 (68.9%)	326 (67.9%)	281 (69.7%)	45 (58.4%)	0.07
Study phase	**939**	**480**	**403**	**77**	
Phase 0	6 (0.6%)	5 (1.0%)	3 (0.7%)	2 (2.6%)	0.14
Phase 1	252 (26.8%)	132 (27.5%)	108 (26.8%)	24 (31.2%)	0.43
Phase 1/Phase 2	342 (36.4%)	194 (40.4%)	162 (40.2%)	32 (41.6%)	0.82
Phase 2	187 (19.9%)	92 (19.2%)	80 (19.9%)	12 (15.6%)	0.38
Phase 2/Phase 3	38 (4.0%)	16 (3.3%)	16 (4.0%)	0 (0.0%)	0.08
Phase 3	41 (4.3%)	20 (4.2%)	16 (4.0%)	4 (5.2%)	0.62
N/A	73 (7.8%)	21 (4.4%)	18 (4.4%)	3 (3.9%)	0.82
Area of expertise	**924**	**474**	**400**	**74**	
Bacterial Infections and Mycoses	5 (0.5%)	5 (1.1%)	4 (1.0%)	1 (1.4%)	0.79
Virus Diseases	3 (0.3%)	3 (0.6%)	3 (0.5%)	1 (1.4%)	0.40
Parasitic Diseases	1 (0.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	-
Neoplasms	12 (1.3%)	6 (1.3%)	5 (1.3%)	1 (1.4%)	0.94
Musculoskeletal Diseases	110 (11.9%)	66 (13.9%)	54 (13.5%)	12 (16.2%)	0.54
Digestive System Diseases	85 (9.2%)	61 (12.9%)	47 (11.8%)	14 (18.9%)	0.09
Stomatognathic Diseases	10 (1.1%)	10 (2.1%)	9 (2.3%)	1 (1.4%)	0.62
Respiratory Tract Diseases	40 (4.3%)	29 (6.1%)	26 (6.5%)	3 (4.1%)	0.42
Otorhinolaryngologic Diseases	3 (0.3%)	1 (0.2%)	1 (0.3%)	0 (0.0%)	0.67
Nervous System Diseases – [NSD]	195 (21.1%)	96 (20.3%)	82 (20.5%)	14 (18.9%)	0.76
Eye Diseases	43 (4.7%)	11 (2.3%)	10 (2.5%)	1 (1.4%)	0.55
Male Urogenital Diseases	50 (5.4%)	26 (5.5%)	20 (5.0%)	6 (8.1%)	0.28
Female Urogenital Diseases and Pregnancy Complications	47 (5.1%)	27 (5.7%)	19 (4.8%)	8 (10.8%)	0.04 *
Cardiovascular diseases – [CVD]	274 (29.7%)	86 (18.1%)	75 (18.8%)	11 (14.9%)	0.43
Hemic and Lymphatic Diseases	21 (2.3%)	15 (3.2%)	14 (3.5%)	1 (1.4%)	0.33
Congenital, Hereditary, and Neonatal Diseases and Abnormalities	58 (6.3%)	26 (5.5%)	24 (6.0%)	2 (2.7%)	0.25
Skin and Connective Tissue Diseases	52 (5.6%)	34 (7.2%)	30 (7.5%)	4 (5.4%)	0.52
Nutritional and Metabolic Diseases	64 (6.9%)	29 (6.1%)	27 (6.8%)	2 (2.7%)	0.18
Endocrine System Diseases	49 (5.3%)	28 (5.9%)	25 (6.3%)	3 (4.1%)	0.46
Immune System Diseases – [ISD]	109 (11.8%)	91 (19.2%)	85 (21.3%)	6 (8.1%)	0.01 **
Wounds and injuries	91 (9.8%)	53 (11.2%)	40 (10.0%)	13 (17.6%)	0.06

Supplementary Table 2.
Characteristics of trials.

Results for trials excluding therapeutics based on hematopoietic stem cell transplant, for trials using mesenchymal stromal cells including ASCs (MSCs), for trials using MSCs excluding ASCs, and for trials using adipose-derived stromal cells (ASCs). Characteristics were statistically compared between studies with ASCs and studies using MSCs (excluding ASCs) by means of a Chi^2 test for qualitative outcomes. Significance is presented as follows: * $p<0.5$, ** $p<0.01$, *** $p<0.001$. Median and interquartile ranges are provided for study sizes retrieved from included trials, and frequencies and percentage are provided for categorical outcomes. State of the study, gender, country of origin, blinding, randomization, type of stem cells employed, and cell donors are described. The probable funding source was derived using an algorithm furnished by Califf *et al.*, and is categorized as NIH, industry or others.

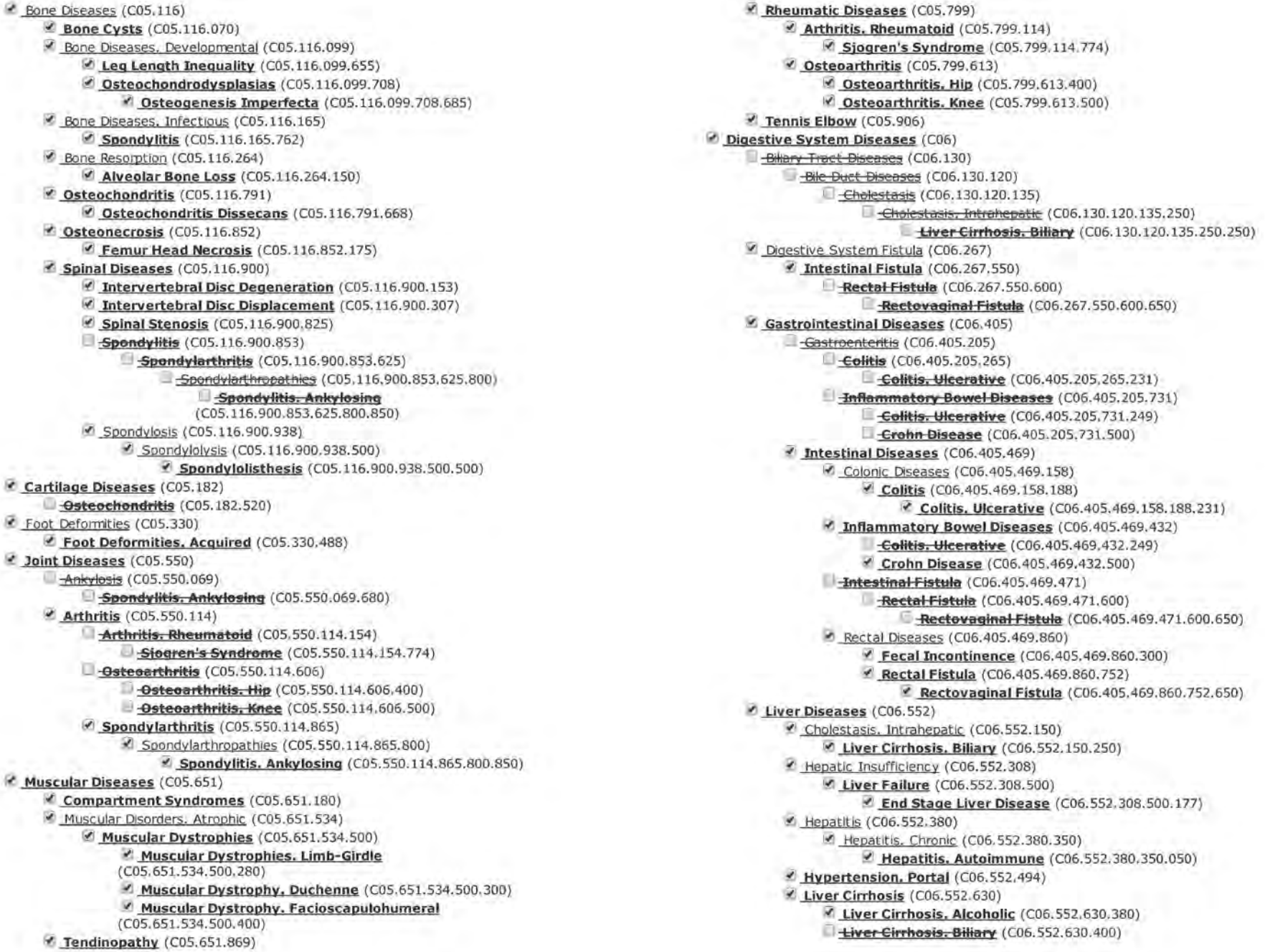
Name of product	Manufacturer	Type of cells	Type of graft	Studies
AC607	Allocure	BM-MSC	Allogeneic	1
Adipocell/ANTG-adip	Bioheart	Adipocytes derived-ASC	Autologous	1
Adipoplus	Anterogen	ASC	Autologous	2
ALD301/401/451	Aldagen/Cytomedix	Selected BM-MNC	Autologous	3
ALLO-ASC	Anterogen	ASC	Allogeneic	4
Allostem	Allosource	ASC	Allogeneic	1
AMR-001/NBS10	Neostem/Caladrius	CD34+ cells	Autologous	2
ANT-SM/ANTG ASC	Anterogen	ASC	Autologous	2
ASCT01	Lifecells	BM-MNC	Autologous	1
C3 BS-CQR-1	Cardio3 BioSciences	MSC-CC	Autologous	2
Cartistem	Medipost	UC-MSC	Allogeneic	3
C-Cure	Cardio3 BioSciences	MSC-CC	Autologous	1
Cellbeads (GLP-1)	CellMed AG/BTG	MSC	Allogeneic	1
CAP-1002	Capricor	CDC	Allogeneic	2
CEP-41750	Teva	MPC	Allogeneic	1
Cerecellgram-Spine	Pharmicell	BM-MSC	Autologous	1
Cerecellgram-ED	Pharmicell	BM-MSC	Autologous	1
Chondrogen	Mesoblast	BM-MSC	Allogeneic	2
Cordstem-ST	CHABiotech	UC-MSC	Allogeneic	1
CTX0E03	ReNeuron	NSC w/ genetic modifications	Allogeneic	3
CX401 eASC	Cellerix/TiGenix	ASC	Autologous	3
CX601	Cellerix/TiGenix	ASC	Allogeneic	4
CX611-0101 eASC	Cellerix/TiGenix	ASC	Allogeneic	2
Furestem-AD	Kang Stem Biotech	UC-MSC	Allogeneic	1
Furestem-CD	Kang Stem Biotech	UC-MSC	Allogeneic	1
Furestem-RA	Kang Stem Biotech	UC-MSC	Allogeneic	1
Hearticellgram-AMI	Pharmicell	BM-MSC	Autologous	1
Hepastem	Promethera	Liver-P	Allogeneic	1
HomeoGH	HomeoTherapy	BM-MSC	Allogeneic	1
HuCNS SC	StemCells	NSC	Allogeneic	6
HYNR-CS Inj	Corestem	BM-MSC	Autologous	2
HYNR-CS-AlloInj	Corestem	BM-MSC	Allogeneic	1
Ixmyelocel-T	Vericel	BM-MSC	Autologous	2
Livercellgram	Pharmicell	BM-MSC	Autologous	1
Lungcellgram	Pharmicell	BM-MSC	Autologous	1
MA09-hRPE	Ocata therapeutics	hESC-RPE	Allogeneic	6
Mesendo	TCA Cellular Therapy	BM-MSC and EPC	Autologous	5
MSB-CAR001	Mesoblast	BM-MSC	Allogeneic	1
MSC-Apceth	Apceth GmbH	BM-MSC	Autologous	1
MSC-NTF/Nurown	Brainstorm Cell therapeutics	BM-MSC w/ growth factors	Autologous	2
Multistem	Pfizer/Athersys	MPC	Allogeneic	4
Myelocell	Bioheart	musc-SC	Autologous	1
Neofuse	Mesoblast	MPC	Allogeneic	5
Neurostem-AD	Medipost	UC-MSC	Allogeneic	2
Nucel	Nutech	Amniotic-SC	Allogeneic	1
cenplacel-L/PDA001	Celgene	pl-MSC	Allogeneic	3
PF-05206388	Pfizer	hESC-RPE	Allogeneic	1
PLX-PAD	Pluristem	pl-MSC	Allogeneic	3
Pneumostem	Medipost	UC-MSC	Allogeneic	3
Map3	RTI Biologics	MPC	Allogeneic	1
opRegen	CellCure Neurosciences	hESC-RPE	Allogeneic	1
Prochymal/Remestemcel	Osiris Therapeutics	BM-MSC	Allogeneic	13
Promostem	Medipost	UC-MSC	Allogeneic	1
Provacel	Osiris Therapeutics	BM-MSC	Allogeneic	1
Repaircell/ANL-adip-AL	Anterogen	Adipocytes derived-ASC	Allogeneic	1
Revascor	Mesoblast	MPC	Allogeneic	3
RNL-Astrostem	K-Stemcell	ASC	Autologous	1
RNL-Jointstem	K-Stemcell	ASC	Autologous	1
RNL-Vascostem	K-Stemcell	ASC	Autologous	1
SB623	SanBio	BM-MSC w/ genetic modification	Allogeneic	1
Stempeucel	Stempeutics	BM-MSC	Allogeneic	1
Trinity Evolution	Orthofix	BM-MSC and OP	Allogeneic	2
Vescell	Theravitae	APC	Autologous	4
XCEL-M-ALPHA	XCelia	BM-MSC	Autologous	2
XCEL-MT-OSTEO-ALPHA	XCelia	BM-MSC	Autologous	3

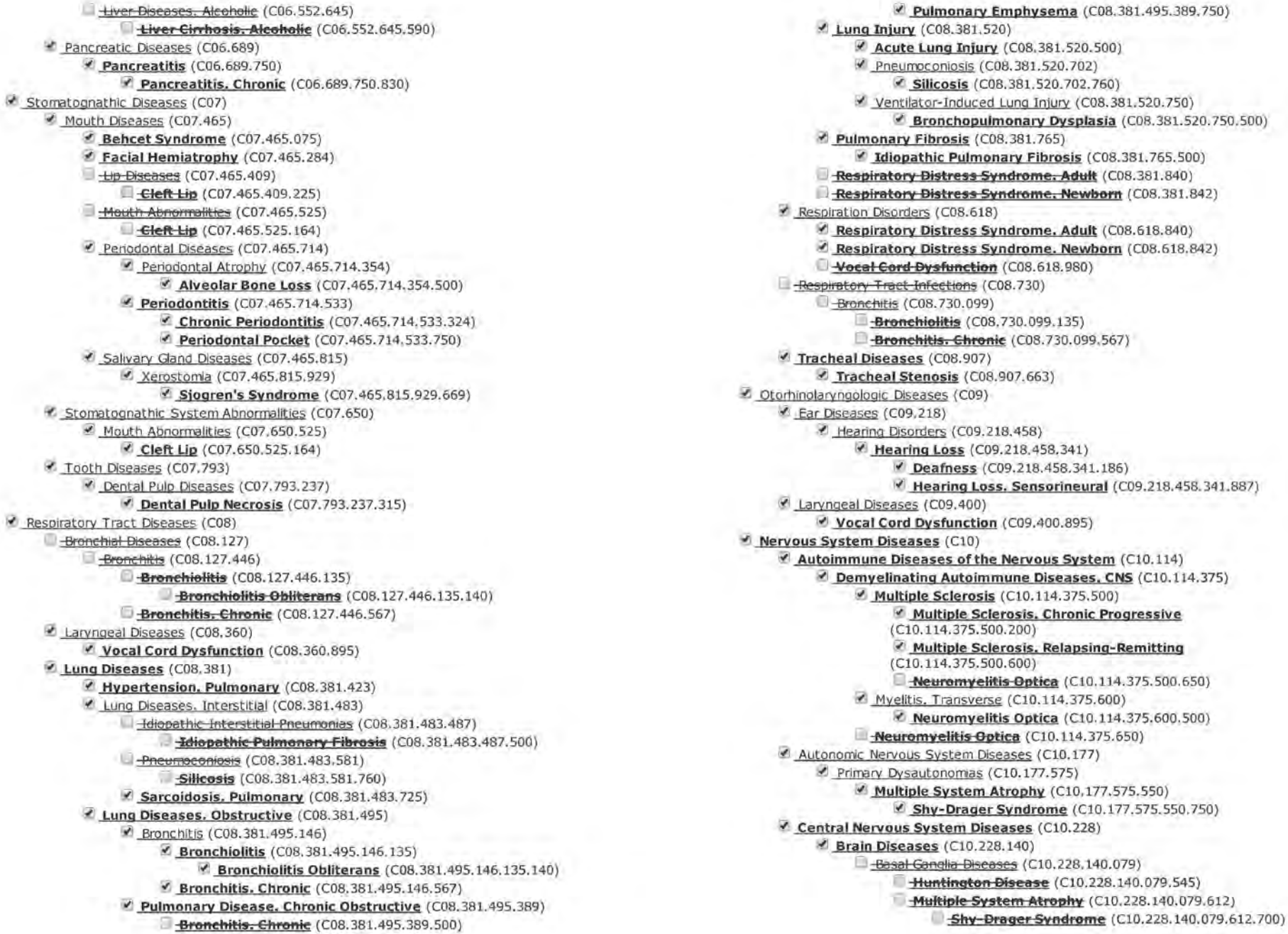
Supplementary Table 3.

List of cellular specialties identified during screening, with the description of the type of cells (cellular source, allogeneic or autologous), and the number of trials.

Supplementary File S1

- Bacterial Infections and Mycoses (C01)
  - Infection (C01.539)
    - Bone Diseases, Infectious (C01.539.160)
      - **Spondylitis** (C01.539.160.762)
    - **Sepsis** (C01.539.757)
      - **Shock, Septic** (C01.539.757.800)
- Virus Diseases (C02)
  - DNA Virus Infections (C02.256)
    - Herpesviridae Infections (C02.256.466)
      - **Cytomegalovirus Infections** (C02.256.466.245)
  - ~~RNA Virus Infections~~ (C02.782)
    - ~~Retroviridae Infections~~ (C02.782.815)
      - ~~Lentivirus Infections~~ (C02.782.815.616)
        - ~~**HIV Infections**~~ (C02.782.815.616.400)
          - ~~**Acquired Immunodeficiency Syndrome**~~ (C02.782.815.616.400.040)
  - Sexually Transmitted Diseases (C02.800)
    - Sexually Transmitted Diseases, Viral (C02.800.801)
      - **HIV Infections** (C02.800.801.400)
        - **Acquired Immunodeficiency Syndrome** (C02.800.801.400.040)
  - ~~Slow Virus Diseases~~ (C02.839)
    - ~~**Acquired Immunodeficiency Syndrome**~~ (C02.839.040)
- Parasitic Diseases (C03)
  - Protozoan Infections (C03.752)
    - Euglenozoa Infections (C03.752.300)
      - Trypanosomiasis (C03.752.300.900)
        - Chagas Disease (C03.752.300.900.200)
          - **Chagas Cardiomyopathy** (C03.752.300.900.200.190)
- Neoplasms (C04)
  - Cysts (C04.182)
    - **Bone Cysts** (C04.182.089)
  - Neoplasms by Histologic Type (C04.557)
    - ~~Neoplasms, Germ Cell and Embryonal~~ (C04.557.465)
      - ~~Neuroectodermal Tumors~~ (C04.557.465.625)
        - ~~Neoplasms, Neuroepithelial~~ (C04.557.465.625.600)
          - ~~**Glioma**~~ (C04.557.465.625.600.380)
            - ~~**Astrocytoma**~~ (C04.557.465.625.600.380.080)
              - ~~**Glioblastoma**~~ (C04.557.465.625.600.380.080.335)
            - ~~**Gliosarcoma**~~ (C04.557.465.625.600.380.400)
            - ~~**Oligodendroglioma**~~ (C04.557.465.625.600.380.590)
    - Neoplasms, Glandular and Epithelial (C04.557.470)
      - **Carcinoma** (C04.557.470.200)
        - **Adenocarcinoma** (C04.557.470.200.025)
          - **Carcinoma, Ductal** (C04.557.470.200.025.232)
          - **Carcinoma, Ductal, Breast** (C04.557.470.200.025.232.500)
          - ~~**Carcinoma, Endometrioid**~~ (C04.557.470.200.025.240)
          - **Cystadenocarcinoma** (C04.557.470.200.025.480)
            - **Cystadenocarcinoma, Mucinous** (C04.557.470.200.025.480.225)
            - **Cystadenocarcinoma, Serous** (C04.557.470.200.025.480.240)
    - ~~Neoplasms, Cystic, Mucinous, and Serous~~ (C04.557.470.590)
      - ~~**Cystadenocarcinoma**~~ (C04.557.470.590.480)
        - ~~**Cystadenocarcinoma, Mucinous**~~ (C04.557.470.590.480.225)
        - ~~**Cystadenocarcinoma, Serous**~~ (C04.557.470.590.480.240)
    - ~~Neoplasms, Ductal, Lobular, and Medullary~~ (C04.557.470.615)
      - ~~**Carcinoma, Ductal**~~ (C04.557.470.615.132)
        - ~~**Carcinoma, Ductal, Breast**~~ (C04.557.470.615.132.500)
    - ~~Neoplasms, Neuroepithelial~~ (C04.557.470.670)
      - ~~**Glioma**~~ (C04.557.470.670.380)
        - ~~**Astrocytoma**~~ (C04.557.470.670.380.080)
          - ~~**Glioblastoma**~~ (C04.557.470.670.380.080.335)
        - ~~**Gliosarcoma**~~ (C04.557.470.670.380.400)
        - ~~**Oligodendroglioma**~~ (C04.557.470.670.380.590)
  - Neoplasms, Nerve Tissue (C04.557.580)
    - Neuroectodermal Tumors (C04.557.580.625)
      - Neoplasms, Neuroepithelial (C04.557.580.625.600)
        - **Glioma** (C04.557.580.625.600.380)
          - **Astrocytoma** (C04.557.580.625.600.380.080)
            - **Glioblastoma** (C04.557.580.625.600.380.080.335)
          - **Gliosarcoma** (C04.557.580.625.600.380.400)
          - **Oligodendroglioma** (C04.557.580.625.600.380.590)
  - Neoplasms by Site (C04.588)
    - ~~Breast Neoplasms~~ (C04.588.180)
      - ~~**Carcinoma, Ductal, Breast**~~ (C04.588.180.390)
    - **Head and Neck Neoplasms** (C04.588.443)
    - Nervous System Neoplasms (C04.588.614)
      - Central Nervous System Neoplasms (C04.588.614.250)
        - **Brain Neoplasms** (C04.588.614.250.195)
    - Urogenital Neoplasms (C04.588.945)
      - Genital Neoplasms, Female (C04.588.945.418)
        - Uterine Neoplasms (C04.588.945.418.948)
          - Endometrial Neoplasms (C04.588.945.418.948.585)
            - **Carcinoma, Endometrioid** (C04.588.945.418.948.585.124)
      - Genital Neoplasms, Male (C04.588.945.440)
        - **Prostatic Neoplasms** (C04.588.945.440.770)
    - Precancerous Conditions (C04.834)
      - **Preleukemia** (C04.834.770)
- Musculoskeletal Diseases (C05)


Bone Diseases (C05.116)
Bone Cysts (C05.116.070)
Bone Diseases, Developmental (C05.116.099)
Leg Length Inequality (C05.116.099.655)
Osteochondrodysplasias (C05.116.099.708)
Osteogenesis Imperfecta (C05.116.099.708.685)
Bone Diseases, Infectious (C05.116.165)
Spondylitis (C05.116.165.762)
Bone Resorption (C05.116.264)
Alveolar Bone Loss (C05.116.264.150)
Osteochondritis (C05.116.791)
Osteochondritis Dissecans (C05.116.791.668)
Osteonecrosis (C05.116.852)
Femur Head Necrosis (C05.116.852.175)
Spinal Diseases (C05.116.900)
Intervertebral Disc Degeneration (C05.116.900.153)
Intervertebral Disc Displacement (C05.116.900.307)
Spinal Stenosis (C05.116.900.825)
Spondylitis (C05.116.900.853)
Spondylarthritis (C05.116.900.853.625)
Spondylarthropathies (C05.116.900.853.625.800)
Spondylitis, Ankylosing (C05.116.900.853.625.800.850)
Spondylosis (C05.116.900.938)
Spondylolysis (C05.116.900.938.500)
Spondylolisthesis (C05.116.900.938.500.500)
Cartilage Diseases (C05.182)
Osteochondritis (C05.182.520)
Foot Deformities (C05.330)
Foot Deformities, Acquired (C05.330.488)
Joint Diseases (C05.550)
Ankylosis (C05.550.069)
Spondylitis, Ankylosing (C05.550.069.680)
Arthritis (C05.550.114)
Arthritis, Rheumatoid (C05.550.114.154)
Sjogren's Syndrome (C05.550.114.154.774)
Osteoarthritis (C05.550.114.606)
Osteoarthritis, Hip (C05.550.114.606.400)
Osteoarthritis, Knee (C05.550.114.606.500)
Spondylarthritis (C05.550.114.865)
Spondylarthropathies (C05.550.114.865.800)
Spondylitis, Ankylosing (C05.550.114.865.800.850)
Muscular Diseases (C05.651)
Compartment Syndromes (C05.651.180)
Muscular Disorders, Atrophic (C05.651.534)
Muscular Dystrophies (C05.651.534.500)
Muscular Dystrophies, Limb-Girdle (C05.651.534.500.280)
Muscular Dystrophy, Duchenne (C05.651.534.500.300)
Muscular Dystrophy, Facioscapulohumeral (C05.651.534.500.400)
Tendinopathy (C05.651.869)
Rheumatic Diseases (C05.799)
Arthritis, Rheumatoid (C05.799.114)
Sjogren's Syndrome (C05.799.114.774)
Osteoarthritis (C05.799.613)
Osteoarthritis, Hip (C05.799.613.400)
Osteoarthritis, Knee (C05.799.613.500)
Tennis Elbow (C05.906)
Digestive System Diseases (C06)
Biliary Tract Diseases (C06.130)
Bile Duct Diseases (C06.130.120)
Cholestasis (C06.130.120.135)
Cholestasis, Intrahepatic (C06.130.120.135.250)
Liver Cirrhosis, Biliary (C06.130.120.135.250.250)
Digestive System Fistula (C06.267)
Intestinal Fistula (C06.267.550)
Rectal Fistula (C06.267.550.600)
Rectovaginal Fistula (C06.267.550.600.650)
Gastrointestinal Diseases (C06.405)
Gastroenteritis (C06.405.205)
Colitis (C06.405.205.265)
Colitis, Ulcerative (C06.405.205.265.231)
Inflammatory Bowel Diseases (C06.405.205.731)
Colitis, Ulcerative (C06.405.205.731.249)
Crohn Disease (C06.405.205.731.500)
Intestinal Diseases (C06.405.469)
Colonic Diseases (C06.405.469.158)
Colitis (C06.405.469.158.188)
Colitis, Ulcerative (C06.405.469.158.188.231)
Inflammatory Bowel Diseases (C06.405.469.432)
Colitis, Ulcerative (C06.405.469.432.249)
Crohn Disease (C06.405.469.432.500)
Intestinal Fistula (C06.405.469.471)
Rectal Fistula (C06.405.469.471.600)
Rectovaginal Fistula (C06.405.469.471.600.650)
Rectal Diseases (C06.405.469.860)
Fecal Incontinence (C06.405.469.860.300)
Rectal Fistula (C06.405.469.860.752)
Rectovaginal Fistula (C06.405.469.860.752.650)
Liver Diseases (C06.552)
Cholestasis, Intrahepatic (C06.552.150)
Liver Cirrhosis, Biliary (C06.552.150.250)
Hepatic Insufficiency (C06.552.308)
Liver Failure (C06.552.308.500)
End Stage Liver Disease (C06.552.308.500.177)
Hepatitis (C06.552.380)
Hepatitis, Chronic (C06.552.380.350)
Hepatitis, Autoimmune (C06.552.380.350.050)
Hypertension, Portal (C06.552.494)
Liver Cirrhosis (C06.552.630)
Liver Cirrhosis, Alcoholic (C06.552.630.380)
Liver Cirrhosis, Biliary (C06.552.630.400)


Liver Diseases, Alcoholic (C06.552.645)
Liver Cirrhosis, Alcoholic (C06.552.645.590)
Pancreatic Diseases (C06.689)
Pancreatitis (C06.689.750)
Pancreatitis, Chronic (C06.689.750.830)
Stomatognathic Diseases (C07)
Mouth Diseases (C07.465)
Behcet Syndrome (C07.465.075)
Facial Hemiatrophy (C07.465.284)
Lip Diseases (C07.465.409)
Cleft Lip (C07.465.409.225)
Mouth Abnormalities (C07.465.525)
Cleft Lip (C07.465.525.164)
Periodontal Diseases (C07.465.714)
Periodontal Atrophy (C07.465.714.354)
Alveolar Bone Loss (C07.465.714.354.500)
Periodontitis (C07.465.714.533)
Chronic Periodontitis (C07.465.714.533.324)
Periodontal Pocket (C07.465.714.533.750)
Salivary Gland Diseases (C07.465.815)
Xerostomia (C07.465.815.929)
Sjogren's Syndrome (C07.465.815.929.669)
Stomatognathic System Abnormalities (C07.650)
Mouth Abnormalities (C07.650.525)
Cleft Lip (C07.650.525.164)
Tooth Diseases (C07.793)
Dental Pulp Diseases (C07.793.237)
Dental Pulp Necrosis (C07.793.237.315)
Respiratory Tract Diseases (C08)
Bronchial Diseases (C08.127)
Bronchitis (C08.127.446)
Bronchiolitis (C08.127.446.135)
Bronchiolitis Obliterans (C08.127.446.135.140)
Bronchitis, Chronic (C08.127.446.567)
Laryngeal Diseases (C08.360)
Vocal Cord Dysfunction (C08.360.895)
Lung Diseases (C08.381)
Hypertension, Pulmonary (C08.381.423)
Lung Diseases, Interstitial (C08.381.483)
Idiopathic Interstitial Pneumonias (C08.381.483.487)
Idiopathic Pulmonary Fibrosis (C08.381.483.487.500)
Pneumoconiosis (C08.381.483.581)
Silicosis (C08.381.483.581.760)
Sarcoidosis, Pulmonary (C08.381.483.725)
Lung Diseases, Obstructive (C08.381.495)
Bronchitis (C08.381.495.146)
Bronchiolitis (C08.381.495.146.135)
Bronchiolitis Obliterans (C08.381.495.146.135.140)
Bronchitis, Chronic (C08.381.495.146.567)
Pulmonary Disease, Chronic Obstructive (C08.381.495.389)
Bronchitis, Chronic (C08.381.495.389.500)
Pulmonary Emphysema (C08.381.495.389.750)
Lung Injury (C08.381.520)
Acute Lung Injury (C08.381.520.500)
Pneumoconiosis (C08.381.520.702)
Silicosis (C08.381.520.702.760)
Ventilator-Induced Lung Injury (C08.381.520.750)
Bronchopulmonary Dysplasia (C08.381.520.750.500)
Pulmonary Fibrosis (C08.381.765)
Idiopathic Pulmonary Fibrosis (C08.381.765.500)
Respiratory Distress Syndrome, Adult (C08.381.840)
Respiratory Distress Syndrome, Newborn (C08.381.842)
Respiration Disorders (C08.618)
Respiratory Distress Syndrome, Adult (C08.618.840)
Respiratory Distress Syndrome, Newborn (C08.618.842)
Vocal Cord Dysfunction (C08.618.980)
Respiratory Tract Infections (C08.730)
Bronchitis (C08.730.099)
Bronchiolitis (C08.730.099.135)
Bronchitis, Chronic (C08.730.099.567)
Tracheal Diseases (C08.907)
Tracheal Stenosis (C08.907.663)
Otorhinolaryngologic Diseases (C09)
Ear Diseases (C09.218)
Hearing Disorders (C09.218.458)
Hearing Loss (C09.218.458.341)
Deafness (C09.218.458.341.186)
Hearing Loss, Sensorineural (C09.218.458.341.887)
Laryngeal Diseases (C09.400)
Vocal Cord Dysfunction (C09.400.895)
Nervous System Diseases (C10)
Autoimmune Diseases of the Nervous System (C10.114)
Demyelinating Autoimmune Diseases, CNS (C10.114.375)
Multiple Sclerosis (C10.114.375.500)
Multiple Sclerosis, Chronic Progressive (C10.114.375.500.200)
Multiple Sclerosis, Relapsing-Remitting (C10.114.375.500.600)
Neuromyelitis Optica (C10.114.375.500.650)
Myelitis, Transverse (C10.114.375.600)
Neuromyelitis Optica (C10.114.375.600.500)
Neuromyelitis Optica (C10.114.375.650)
Autonomic Nervous System Diseases (C10.177)
Primary Dysautonomias (C10.177.575)
Multiple System Atrophy (C10.177.575.550)
Shy-Drager Syndrome (C10.177.575.550.750)
Central Nervous System Diseases (C10.228)
Brain Diseases (C10.228.140)
Basal Ganglia Diseases (C10.228.140.079)
Huntington Disease (C10.228.140.079.545)
Multiple System Atrophy (C10.228.140.079.612)
Shy-Drager Syndrome (C10.228.140.079.612.700)


Parkinsonian Disorders (C10.228.140.079.862)
Parkinson Disease (C10.228.140.079.862.500)
Supranuclear Palsy, Progressive (C10.228.140.079.882)
Brain Damage, Chronic (C10.228.140.140)
Cerebral Palsy (C10.228.140.140.254)
Brain Diseases, Metabolic (C10.228.140.163)
Brain Diseases, Metabolic, Inborn (C10.228.140.163.100)
Hereditary Central Nervous System Demyelinating Diseases (C10.228.140.163.100.362)
Adrenoleukodystrophy (C10.228.140.163.100.362.250)
Pelizaeus-Merzbacher Disease (C10.228.140.163.100.362.775)
Peroxisomal Disorders (C10.228.140.163.100.680)
Adrenoleukodystrophy (C10.228.140.163.100.680.100)
Urea Cycle Disorders, Inborn (C10.228.140.163.100.937)
Brain Injuries (C10.228.140.199)
Brain Neoplasms (C10.228.140.211)
Cerebellar Diseases (C10.228.140.252)
Cerebellar Ataxia (C10.228.140.252.190)
Spinocerebellar Degenerations (C10.228.140.252.700)
Cerebrovascular Disorders (C10.228.140.300)
Brain Ischemia (C10.228.140.300.150)
Brain Infarction (C10.228.140.300.150.477)
Cerebral Infarction (C10.228.140.300.150.477.200)
Infarction, Anterior Cerebral Artery (C10.228.140.300.150.477.200.400)
Infarction, Middle Cerebral Artery (C10.228.140.300.150.477.200.450)
Infarction, Posterior Cerebral Artery (C10.228.140.300.150.477.200.475)
Hypoxia-Ischemia, Brain (C10.228.140.300.150.716)
Intracranial Arterial Diseases (C10.228.140.300.510)
Cerebral Arterial Diseases (C10.228.140.300.510.200)
Infarction, Anterior Cerebral Artery (C10.228.140.300.510.200.325)
Infarction, Middle Cerebral Artery (C10.228.140.300.510.200.387)
Infarction, Posterior Cerebral Artery (C10.228.140.300.510.200.418)
Intracranial Hemorrhages (C10.228.140.300.535)
Cerebral Hemorrhage (C10.228.140.300.535.200)
Stroke (C10.228.140.300.775)
Brain Infarction (C10.228.140.300.775.200)
Cerebral Infarction (C10.228.140.300.775.200.200)
Infarction, Anterior Cerebral Artery (C10.228.140.300.775.200.200.400)
Infarction, Middle Cerebral Artery (C10.228.140.300.775.200.200.450)
Infarction, Posterior Cerebral Artery (C10.228.140.300.775.200.200.475)
Dementia (C10.228.140.380)
Alzheimer Disease (C10.228.140.380.100)
Huntington Disease (C10.228.140.380.278)
Epilepsy (C10.228.140.490)
Epilepsies, Partial (C10.228.140.490.360)
Epilepsy, Temporal Lobe (C10.228.140.490.360.290)
Hypoxia, Brain (C10.228.140.624)
Hypoxia-Ischemia, Brain (C10.228.140.624.500)
Leukoencephalopathies (C10.228.140.695)
Demyelinating Autoimmune Diseases, CNS (C10.228.140.695.562)
Hereditary Central Nervous System Demyelinating Diseases (C10.228.140.695.625)
Adrenoleukodystrophy (C10.228.140.695.625.250)
Pelizaeus-Merzbacher Disease (C10.228.140.695.625.775)
Movement Disorders (C10.228.662)
Dyskinesias (C10.228.662.262)
Chorea (C10.228.662.262.249)
Huntington Disease (C10.228.662.262.249.750)
Multiple System Atrophy (C10.228.662.550)
Shy-Drager Syndrome (C10.228.662.550.700)
Parkinsonian Disorders (C10.228.662.600)
Parkinson Disease (C10.228.662.600.400)
Supranuclear Palsy, Progressive (C10.228.662.700)
Spinal Cord Diseases (C10.228.854)
Amyotrophic Lateral Sclerosis (C10.228.854.139)
Spinal Cord Injuries (C10.228.854.770)
Spinocerebellar Degenerations (C10.228.854.787)
Cranial Nerve Diseases (C10.292)
Facial Nerve Diseases (C10.292.300)
Facial Hemiatrophy (C10.292.300.375)
Ocular Motility Disorders (C10.292.562)
Ophthalmoplegia (C10.292.562.750)
Supranuclear Palsy, Progressive (C10.292.562.750.500)
Optic Nerve Diseases (C10.292.700)
Optic Atrophy (C10.292.700.225)
Optic Neuritis (C10.292.700.550)
Neuromyelitis Optica (C10.292.700.550.500)
Demyelinating Diseases (C10.314)
Demyelinating Autoimmune Diseases, CNS (C10.314.350)
Multiple Sclerosis (C10.314.350.500)
Multiple Sclerosis, Chronic Progressive (C10.314.350.500.200)
Multiple Sclerosis, Relapsing-Remitting (C10.314.350.500.600)
Neuromyelitis Optica (C10.314.350.500.650)
Myelitis, Transverse (C10.314.350.600)
Neuromyelitis Optica (C10.314.350.600.500)

- ~~Neuromyelitis Optica~~ (C10.314.350.650)
- ~~Hereditary Central Nervous System Demyelinating Diseases~~ (C10.314.400)
  - ~~Adrenoleukodystrophy~~ (C10.314.400.250)
  - ~~Pelizaeus-Merzbacher Disease~~ (C10.314.400.775)
- Nervous System Neoplasms (C10.551)
  - Central Nervous System Neoplasms (C10.551.240)
    - **Brain Neoplasms** (C10.551.240.250)
- **Neurodegenerative Diseases** (C10.574)
  - Heredodegenerative Disorders, Nervous System (C10.574.500)
    - **Huntington Disease** (C10.574.500.497)
    - **Neuronal Ceroid-Lipofuscinoses** (C10.574.500.550)
    - **Spinocerebellar Degenerations** (C10.574.500.825)
  - ~~Motor Neuron Disease~~ (C10.574.562)
    - ~~Amyotrophic Lateral Sclerosis~~ (C10.574.562.250)
  - ~~Multiple System Atrophy~~ (C10.574.625)
    - ~~Shy-Drager Syndrome~~ (C10.574.625.700)
  - **Parkinson Disease** (C10.574.812)
  - ~~Shy-Drager Syndrome~~ (C10.574.875)
  - Tauopathies (C10.574.945)
    - **Alzheimer Disease** (C10.574.945.249)
    - **Supranuclear Palsy, Progressive** (C10.574.945.500)
  - TDP-43 Proteinopathies (C10.574.950)
    - **Amyotrophic Lateral Sclerosis** (C10.574.950.050)
- Neurologic Manifestations (C10.597)
  - ~~Dyskinesias~~ (C10.597.350)
    - ~~Ataxia~~ (C10.597.350.090)
      - ~~Cerebellar Ataxia~~ (C10.597.350.090.500)
  - Neurobehavioral Manifestations (C10.597.606)
    - **Intellectual Disability** (C10.597.606.643)
      - ~~Mental Retardation, X-Linked~~ (C10.597.606.643.455)
        - ~~Adrenoleukodystrophy~~ (C10.597.606.643.455.124)
  - Pain (C10.597.617)
    - **Back Pain** (C10.597.617.232)
      - **Low Back Pain** (C10.597.617.232.400)
  - ~~Paralysis~~ (C10.597.622)
    - ~~Ophthalmoplegia~~ (C10.597.622.447)
      - ~~Supranuclear Palsy, Progressive~~ (C10.597.622.447.690)
  - Sensation Disorders (C10.597.751)
    - Hearing Disorders (C10.597.751.418)
      - **Hearing Loss** (C10.597.751.418.341)
        - **Deafness** (C10.597.751.418.341.186)
        - **Hearing Loss, Sensorineural** (C10.597.751.418.341.887)
- **Neuromuscular Diseases** (C10.668)
  - **Motor Neuron Disease** (C10.668.467)
    - ~~Amyotrophic Lateral Sclerosis~~ (C10.668.467.250)
  - **Muscular Diseases** (C10.668.491)
    - Muscular Disorders, Atrophic (C10.668.491.175)
      - **Muscular Dystrophies** (C10.668.491.175.500)
        - **Muscular Dystrophies, Limb-Girdle** (C10.668.491.175.500.149)
        - **Muscular Dystrophy, Duchenne** (C10.668.491.175.500.300)
        - **Muscular Dystrophy, Facioscapulohumeral** (C10.668.491.175.500.400)
  - **Peripheral Nervous System Diseases** (C10.668.829)
    - **Diabetic Neuropathies** (C10.668.829.300)
    - **Peripheral Nerve Injuries** (C10.668.829.712)
- **Trauma, Nervous System** (C10.900)
  - Craniocerebral Trauma (C10.900.300)
    - ~~Brain Injuries~~ (C10.900.300.087)
    - **Skull Fractures** (C10.900.300.918)
  - ~~Peripheral Nerve Injuries~~ (C10.900.575)
  - ~~Spinal Cord Injuries~~ (C10.900.850)
- Eye Diseases (C11)
  - **Corneal Diseases** (C11.204)
    - ~~Corneal Injuries~~ (C11.204.284)
  - ~~Eye Diseases, Hereditary~~ (C11.270)
    - ~~Retinitis Pigmentosa~~ (C11.270.684)
  - **Eye Injuries** (C11.297)
    - **Corneal Injuries** (C11.297.374)
  - Lacrimal Apparatus Diseases (C11.496)
    - Dry Eye Syndromes (C11.496.260)
      - **Sjogren's Syndrome** (C11.496.260.719)
  - Ocular Hypertension (C11.525)
    - **Glaucoma** (C11.525.381)
      - **Glaucoma, Open-Angle** (C11.525.381.407)
  - Ocular Motility Disorders (C11.590)
    - Ophthalmoplegia (C11.590.472)
      - **Supranuclear Palsy, Progressive** (C11.590.472.500)
  - **Optic Nerve Diseases** (C11.640)
    - **Optic Atrophy** (C11.640.451)
    - Optic Neuritis (C11.640.576)
      - **Neuromyelitis Optica** (C11.640.576.695)
  - **Retinal Diseases** (C11.768)
    - **Diabetic Retinopathy** (C11.768.257)
    - **Retinal Artery Occlusion** (C11.768.400)
    - **Retinal Degeneration** (C11.768.585)
      - **Macular Degeneration** (C11.768.585.439)
        - **Geographic Atrophy** (C11.768.585.439.122)
      - **Retinal Dystrophies** (C11.768.585.658)
        - **Retinitis Pigmentosa** (C11.768.585.658.500)
    - **Retinitis** (C11.768.773)
  - Uveal Diseases (C11.941)
    - Uveitis (C11.941.879)
      - Panuveitis (C11.941.879.780)
        - Uveitis, Anterior (C11.941.879.780.880)
          - **Behcet Syndrome** (C11.941.879.780.880.200)
- Male Urogenital Diseases (C12)
  - Genital Diseases, Male (C12.294)
    - ~~Genital Neoplasms, Male~~ (C12.294.260)
      - ~~Prostatic Neoplasms~~ (C12.294.260.750)


Infertility (C12.294.365)
Infertility, Male (C12.294.365.700)
Azoospermia (C12.294.365.700.380)
Penile Diseases (C12.294.494)
Penile Induration (C12.294.494.508)
Prostatic Diseases (C12.294.565)
Prostatic Neoplasms (C12.294.565.625)
Sexual Dysfunction, Physiological (C12.294.644)
Erectile Dysfunction (C12.294.644.486)
Impotence, Vasculogenic (C12.294.644.486.500)
Urogenital Abnormalities (C12.706)
Bladder Exstrophy (C12.706.132)
Disorders of Sex Development (C12.706.316)
Sex Chromosome Disorders of Sex Development (C12.706.316.795)
Klinefelter Syndrome (C12.706.316.795.500)
Multicystic Dysplastic Kidney (C12.706.629)
Urogenital Neoplasms (C12.758)
Genital Neoplasms, Male (C12.758.409)
Prostatic Neoplasms (C12.758.409.750)
Urologic Diseases (C12.777)
Kidney Diseases (C12.777.419)
Diabetic Nephropathies (C12.777.419.192)
Fanconi Syndrome (C12.777.419.250)
Hypertension, Renal (C12.777.419.331)
Hypertension, Renovascular (C12.777.419.331.490)
Kidney Diseases, Cystic (C12.777.419.403)
Multicystic Dysplastic Kidney (C12.777.419.403.750)
Polycystic Kidney Diseases (C12.777.419.403.875)
Nephritis (C12.777.419.570)
Glomerulonephritis (C12.777.419.570.363)
Glomerulosclerosis, Focal Segmental (C12.777.419.570.363.660)
Lupus Nephritis (C12.777.419.570.363.680)
Nephrosis (C12.777.419.630)
Nephrosis, Lipoid (C12.777.419.630.477)
Nephrotic Syndrome (C12.777.419.630.643)
Renal Artery Obstruction (C12.777.419.775)
Renal Insufficiency (C12.777.419.780)
Acute Kidney Injury (C12.777.419.780.050)
Kidney Tubular Necrosis, Acute (C12.777.419.780.050.500)
Renal Insufficiency, Chronic (C12.777.419.780.750)
Kidney Failure, Chronic (C12.777.419.780.750.500)
Renal Tubular Transport, Inborn Errors (C12.777.419.815)
Fanconi Syndrome (C12.777.419.815.450)
Urethral Diseases (C12.777.767)
Urethral Obstruction (C12.777.767.700)
Urethral Stricture (C12.777.767.700.700)
Urinary Bladder Diseases (C12.777.829)
Bladder Exstrophy (C12.777.829.132)
Cystitis (C12.777.829.495)


Urination Disorders (C12.777.934)
Urinary Incontinence (C12.777.934.852)
Urinary Incontinence, Stress (C12.777.934.852.249)
Female Urogenital Diseases and Pregnancy Complications (C13)
Female Urogenital Diseases (C13.351)
Genital Diseases, Female (C13.351.500)
Adnexal Diseases (C13.351.500.056)
Ovarian Diseases (C13.351.500.056.630)
Menopause, Premature (C13.351.500.056.630.250)
Ovarian Neoplasms (C13.351.500.056.630.705)
Carcinoma, Endometrioid (C13.351.500.056.630.705.331)
Primary Ovarian Insufficiency (C13.351.500.056.630.750)
Gynatresia (C13.351.500.320)
Infertility (C13.351.500.365)
Infertility, Female (C13.351.500.365.700)
Vaginal Diseases (C13.351.500.894)
Vaginal Fistula (C13.351.500.894.767)
Rectovaginal Fistula (C13.351.500.894.767.249)
Urogenital Abnormalities (C13.351.875)
Bladder Exstrophy (C13.351.875.132)
Disorders of Sex Development (C13.351.875.253)
Sex Chromosome Disorders of Sex Development (C13.351.875.253.795)
Klinefelter Syndrome (C13.351.875.253.795.500)
Multicystic Dysplastic Kidney (C13.351.875.558)
Urogenital Neoplasms (C13.351.937)
Genital Neoplasms, Female (C13.351.937.418)
Ovarian Neoplasms (C13.351.937.418.685)
Carcinoma, Endometrioid (C13.351.937.418.685.331)
Uterine Neoplasms (C13.351.937.418.875)
Endometrial Neoplasms (C13.351.937.418.875.200)
Carcinoma, Endometrioid (C13.351.937.418.875.200.124)
Urologic Diseases (C13.351.968)
Kidney Diseases (C13.351.968.419)
Diabetic Nephropathies (C13.351.968.419.192)
Fanconi Syndrome (C13.351.968.419.250)
Hypertension, Renal (C13.351.968.419.331)
Hypertension, Renovascular (C13.351.968.419.331.490)
Kidney Diseases, Cystic (C13.351.968.419.403)
Multicystic Dysplastic Kidney (C13.351.968.419.403.750)
Polycystic Kidney Diseases (C13.351.968.419.403.875)
Nephritis (C13.351.968.419.570)
Glomerulonephritis (C13.351.968.419.570.363)
Glomerulosclerosis, Focal Segmental (C13.351.968.419.570.363.640)
Lupus Nephritis

- (C13.351.968.419.570.363.680)
- Nephrosis (C13.351.968.419.630)
  - **Nephrosis, Lipoid** (C13.351.968.419.630.477)
  - **Nephrotic Syndrome** (C13.351.968.419.630.643)
- **Renal Artery Obstruction** (C13.351.968.419.775)
- **Renal Insufficiency** (C13.351.968.419.780)
  - **Acute Kidney Injury** (C13.351.968.419.780.050)
    - **Kidney Tubular Necrosis, Acute** (C13.351.968.419.780.050.500)
  - **Renal Insufficiency, Chronic** (C13.351.968.419.780.750)
    - **Kidney Failure, Chronic** (C13.351.968.419.780.750.500)
- Renal Tubular Transport, Inborn Errors (C13.351.968.419.815)
  - **Fanconi Syndrome** (C13.351.968.419.815.450)
- Urethral Diseases (C13.351.968.767)
  - Urethral Obstruction (C13.351.968.767.700)
    - **Urethral Stricture** (C13.351.968.767.700.700)
- Urinary Bladder Diseases (C13.351.968.829)
  - ~~**Bladder Exstrophy**~~ (C13.351.968.829.132)
  - **Cystitis** (C13.351.968.829.495)
- Urination Disorders (C13.351.968.934)
  - **Urinary Incontinence** (C13.351.968.934.814)
    - **Urinary Incontinence, Stress** (C13.351.968.934.814.500)

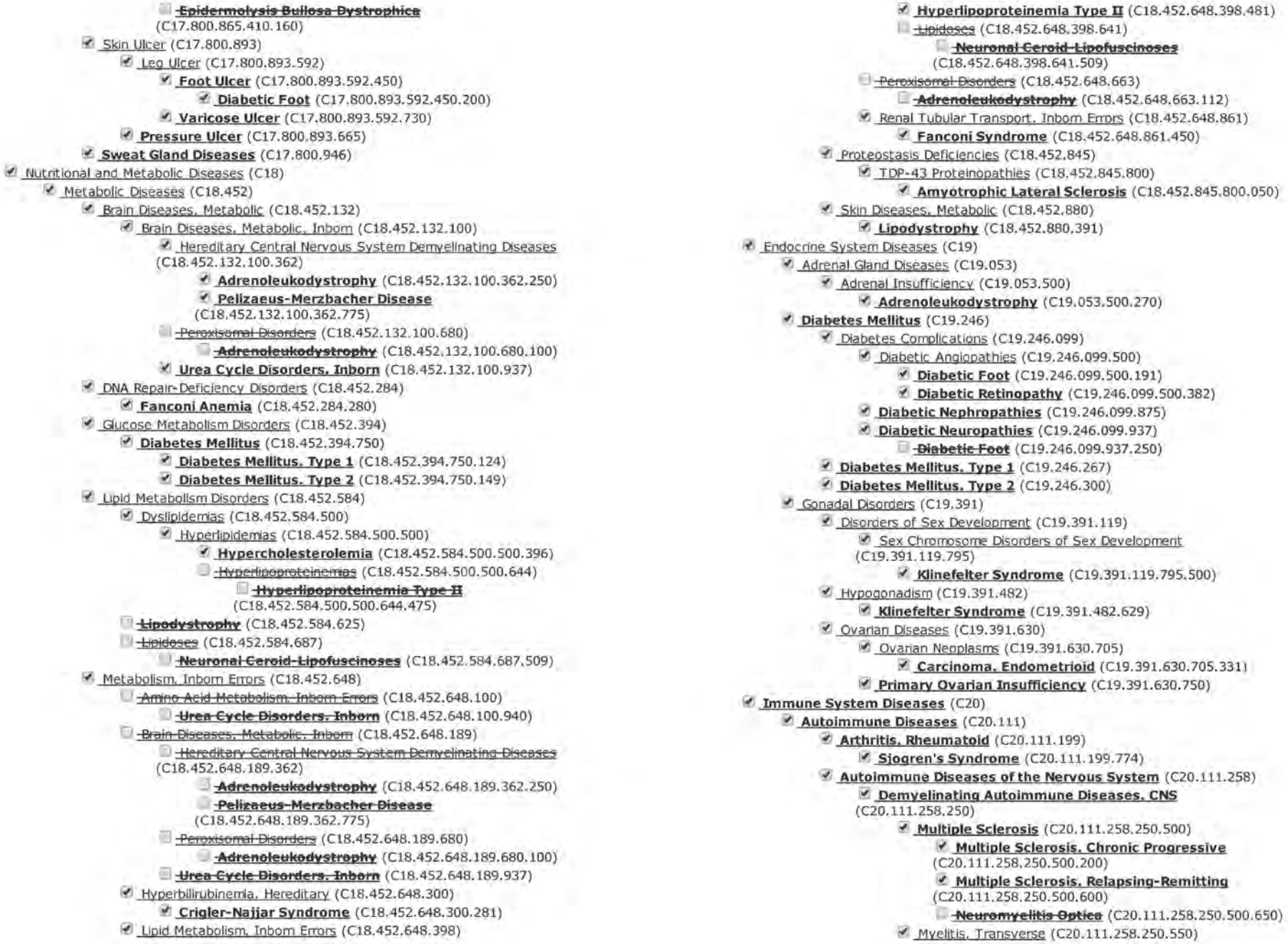
- **Cardiovascular Diseases** (C14)
  - Cardiovascular Abnormalities (C14.240)
    - Heart Defects, Congenital (C14.240.400)
      - **Cor Triatriatum** (C14.240.400.200)
      - **Hypoplastic Left Heart Syndrome** (C14.240.400.625)
  - **Heart Diseases** (C14.280)
    - ~~Cardiomegaly~~ (C14.280.195)
      - ~~**Cardiomyopathy, Dilated**~~ (C14.280.195.160)
    - **Cardiomyopathies** (C14.280.238)
      - **Cardiomyopathy, Dilated** (C14.280.238.070)
      - **Chagas Cardiomyopathy** (C14.280.238.190)
    - ~~Heart Defects, Congenital~~ (C14.280.400)
      - ~~**Cor Triatriatum**~~ (C14.280.400.200)
      - ~~**Hypoplastic Left Heart Syndrome**~~ (C14.280.400.625)
    - **Heart Failure** (C14.280.434)
    - **Myocardial Ischemia** (C14.280.647)
      - **Angina Pectoris** (C14.280.647.187)
        - **Angina, Stable** (C14.280.647.187.362)
      - **Coronary Disease** (C14.280.647.250)
        - **Coronary Artery Disease** (C14.280.647.250.260)
        - **Coronary Occlusion** (C14.280.647.250.272)
      - **Myocardial Infarction** (C14.280.647.500)
        - **Anterior Wall Myocardial Infarction** (C14.280.647.500.093)
    - **Ventricular Dysfunction** (C14.280.945)
      - **Ventricular Dysfunction, Left** (C14.280.945.900)

- **Vascular Diseases** (C14.907)
  - **Arterial Occlusive Diseases** (C14.907.137)
    - **Arteriosclerosis** (C14.907.137.126)
      - **Atherosclerosis** (C14.907.137.126.307)
        - ~~**Peripheral Arterial Disease**~~ (C14.907.137.126.307.500)
      - ~~**Coronary Artery Disease**~~ (C14.907.137.126.339)
      - **Intermittent Claudication** (C14.907.137.126.669)
    - **Renal Artery Obstruction** (C14.907.137.727)
    - **Retinal Artery Occlusion** (C14.907.137.780)
    - ~~**Thromboangiitis Obliterans**~~ (C14.907.137.870)
  - Cerebrovascular Disorders (C14.907.253)
    - **Brain Ischemia** (C14.907.253.092)
      - ~~Brain Infarction~~ (C14.907.253.092.477)
        - ~~**Cerebral Infarction**~~ (C14.907.253.092.477.200)
          - ~~**Infarction, Anterior Cerebral Artery**~~ (C14.907.253.092.477.200.400)
          - ~~**Infarction, Middle Cerebral Artery**~~ (C14.907.253.092.477.200.450)
          - ~~**Infarction, Posterior Cerebral Artery**~~ (C14.907.253.092.477.200.475)
      - **Hypoxia-Ischemia, Brain** (C14.907.253.092.716)
    - ~~Intracranial Arterial Diseases~~ (C14.907.253.560)
      - ~~Cerebral Arterial Diseases~~ (C14.907.253.560.200)
        - ~~**Infarction, Anterior Cerebral Artery**~~ (C14.907.253.560.200.325)
        - ~~**Infarction, Middle Cerebral Artery**~~ (C14.907.253.560.200.387)
        - ~~**Infarction, Posterior Cerebral Artery**~~ (C14.907.253.560.200.418)
    - Intracranial Hemorrhages (C14.907.253.573)
      - **Cerebral Hemorrhage** (C14.907.253.573.200)
    - **Stroke** (C14.907.253.855)
      - Brain Infarction (C14.907.253.855.200)
        - **Cerebral Infarction** (C14.907.253.855.200.200)
          - **Infarction, Anterior Cerebral Artery** (C14.907.253.855.200.200.400)
          - **Infarction, Middle Cerebral Artery** (C14.907.253.855.200.200.450)
          - **Infarction, Posterior Cerebral Artery** (C14.907.253.855.200.200.475)
  - **Compartment Syndromes** (C14.907.303)
  - Diabetic Angiopathies (C14.907.320)
    - **Diabetic Foot** (C14.907.320.191)
    - **Diabetic Retinopathy** (C14.907.320.382)
  - **Hypertension** (C14.907.489)
    - Hypertension, Renal (C14.907.489.631)
      - **Hypertension, Renovascular** (C14.907.489.631.485)
  - Hypotension (C14.907.514)
    - **Shy-Drager Syndrome** (C14.907.514.741)
  - ~~**Myocardial Ischemia**~~ (C14.907.585)
    - ~~**Angina Pectoris**~~ (C14.907.585.187)
      - ~~**Angina, Stable**~~ (C14.907.585.187.362)

- ~~Coronary Disease~~ (C14.907.585.250)
  - ~~Coronary Artery Disease~~ (C14.907.585.250.260)
  - ~~Coronary Occlusion~~ (C14.907.585.250.272)
- ~~Myocardial Infarction~~ (C14.907.585.500)
  - ~~Anterior Wall Myocardial Infarction~~ (C14.907.585.500.093)
- Peripheral Vascular Diseases (C14.907.617)
  - Peripheral Arterial Disease (C14.907.617.671)
- Varicose Veins (C14.907.927)
  - Varicose Ulcer (C14.907.927.730)
- Vasculitis (C14.907.940)
  - Behcet Syndrome (C14.907.940.100)
  - Thromboangiitis Obliterans (C14.907.940.905)

- Hemic and Lymphatic Diseases (C15)
  - Hematologic Diseases (C15.378)
    - Anemia (C15.378.071)
      - Anemia, Aplastic (C15.378.071.085)
        - Anemia, Hypoplastic, Congenital (C15.378.071.085.080)
          - Fanconi Anemia (C15.378.071.085.080.280)
      - ~~Anemia, Hemolytic~~ (C15.378.071.141)
        - ~~Anemia, Hemolytic, Congenital~~ (C15.378.071.141.150)
          - ~~Anemia, Sickle Cell~~ (C15.378.071.141.150.150)
    - Blood Coagulation Disorders (C15.378.100)
      - Blood Coagulation Disorders, Inherited (C15.378.100.100)
        - Hemophilia B (C15.378.100.100.510)
      - ~~Coagulation Protein Disorders~~ (C15.378.100.141)
        - ~~Hemophilia B~~ (C15.378.100.141.510)
    - Blood Platelet Disorders (C15.378.140)
      - Thrombocytopenia (C15.378.140.855)
    - Bone Marrow Diseases (C15.378.190)
      - ~~Anemia, Aplastic~~ (C15.378.190.196)
        - ~~Anemia, Hypoplastic, Congenital~~ (C15.378.190.196.080)
          - ~~Fanconi Anemia~~ (C15.378.190.196.080.280)
      - Myelodysplastic Syndromes (C15.378.190.625)
    - Hemoglobinopathies (C15.378.420)
      - Anemia, Sickle Cell (C15.378.420.155)
    - ~~Hemorrhagic Disorders~~ (C15.378.463)
      - ~~Hemophilia B~~ (C15.378.463.510)
    - Leukocyte Disorders (C15.378.553)
      - Leukopenia (C15.378.553.546)
        - Agranulocytosis (C15.378.553.546.184)
          - Neutropenia (C15.378.553.546.184.564)
    - Pancytopenia (C15.378.700)
    - Preleukemia (C15.378.800)
  - Lymphatic Diseases (C15.604)
    - Lymphedema (C15.604.496)
    - Lymphoproliferative Disorders (C15.604.515)
      - Sarcoidosis (C15.604.515.827)
        - Sarcoidosis, Pulmonary (C15.604.515.827.725)
- Congenital, Hereditary, and Neonatal Diseases and Abnormalities (C16)
  - Congenital Abnormalities (C16.131)
    - ~~Abnormalities, Multiple~~ (C16.131.077)
      - ~~Netherton Syndrome~~ (C16.131.077.619)
    - Cardiovascular Abnormalities (C16.131.240)
      - Heart Defects, Congenital (C16.131.240.400)
        - Cor Triatriatum (C16.131.240.400.200)
        - Hypoplastic Left Heart Syndrome (C16.131.240.400.625)
    - ~~Chromosome Disorders~~ (C16.131.260)
      - ~~Sex Chromosome Disorders~~ (C16.131.260.830)
        - ~~Sex Chromosome Disorders of Sex Development~~ (C16.131.260.830.835)
          - ~~Klinefelter Syndrome~~ (C16.131.260.830.835.500)
    - Skin Abnormalities (C16.131.831)
      - Epidermolysis Bullosa (C16.131.831.493)
        - Epidermolysis Bullosa Dystrophica (C16.131.831.493.160)
      - Ichthyosis (C16.131.831.512)
        - Ichthyosiform Erythroderma, Congenital (C16.131.831.512.400)
          - Netherton Syndrome (C16.131.831.512.400.705)
    - Stomatognathic System Abnormalities (C16.131.850)
      - Mouth Abnormalities (C16.131.850.525)
        - Cleft Lip (C16.131.850.525.164)
    - Urogenital Abnormalities (C16.131.939)
      - Bladder Exstrophy (C16.131.939.132)
      - ~~Disorders of Sex Development~~ (C16.131.939.316)
        - ~~Sex Chromosome Disorders of Sex Development~~ (C16.131.939.316.795)
          - ~~Klinefelter Syndrome~~ (C16.131.939.316.795.500)
      - Multicystic Dysplastic Kidney (C16.131.939.629)
- Genetic Diseases, Inborn (C16.320)
  - ~~Anemia, Hemolytic, Congenital~~ (C16.320.070)
    - ~~Anemia, Sickle Cell~~ (C16.320.070.150)
  - Anemia, Hypoplastic, Congenital (C16.320.077)
    - Fanconi Anemia (C16.320.077.280)
  - Blood Coagulation Disorders, Inherited (C16.320.099)
    - Hemophilia B (C16.320.099.510)
  - Chromosome Disorders (C16.320.180)
    - Sex Chromosome Disorders (C16.320.180.830)
      - Sex Chromosome Disorders of Sex Development (C16.320.180.830.835)
        - Klinefelter Syndrome (C16.320.180.830.835.500)
  - Eye Diseases, Hereditary (C16.320.290)
    - Retinitis Pigmentosa (C16.320.290.684)
  - ~~Genetic Diseases, X-Linked~~ (C16.320.322)
    - ~~Hemophilia B~~ (C16.320.322.235)
    - ~~Mental Retardation, X-Linked~~ (C16.320.322.500)
      - ~~Adrenoleukodystrophy~~ (C16.320.322.500.124)
    - ~~Muscular Dystrophy, Duchenne~~ (C16.320.322.562)
    - ~~Pelizaeus-Merzbacher Disease~~ (C16.320.322.906)
  - Hemoglobinopathies (C16.320.365)
    - Anemia, Sickle Cell (C16.320.365.155)

- Heredodegenerative Disorders, Nervous System (C16.320.400)
  - **Huntington Disease** (C16.320.400.430)
  - ~~Mental Retardation, X-Linked~~ (C16.320.400.525)
    - ~~**Adrenoleukodystrophy**~~ (C16.320.400.525.124)
  - **Neuronal Ceroid-Lipofuscinoses** (C16.320.400.600)
  - **Spinocerebellar Degenerations** (C16.320.400.780)
- Metabolism, Inborn Errors (C16.320.565)
  - ~~Amino Acid Metabolism, Inborn Errors~~ (C16.320.565.100)
    - ~~**Urea Cycle Disorders, Inborn**~~ (C16.320.565.100.940)
  - Brain Diseases, Metabolic, Inborn (C16.320.565.189)
    - Hereditary Central Nervous System Demyelinating Diseases (C16.320.565.189.362)
      - **Adrenoleukodystrophy** (C16.320.565.189.362.250)
      - **Pelizaeus-Merzbacher Disease** (C16.320.565.189.362.775)
    - ~~Peroxisomal Disorders~~ (C16.320.565.189.680)
      - ~~**Adrenoleukodystrophy**~~ (C16.320.565.189.680.100)
    - **Urea Cycle Disorders, Inborn** (C16.320.565.189.937)
  - Hyperbilirubinemia, Hereditary (C16.320.565.300)
    - **Crigler-Najjar Syndrome** (C16.320.565.300.281)
  - Lipid Metabolism, Inborn Errors (C16.320.565.398)
    - **Hyperlipoproteinemia Type II** (C16.320.565.398.481)
    - ~~Lipidoses~~ (C16.320.565.398.641)
      - ~~**Neuronal Ceroid-Lipofuscinoses**~~ (C16.320.565.398.641.509)
  - ~~Peroxisomal Disorders~~ (C16.320.565.663)
    - ~~**Adrenoleukodystrophy**~~ (C16.320.565.663.112)
  - Renal Tubular Transport, Inborn Errors (C16.320.565.861)
    - **Fanconi Syndrome** (C16.320.565.861.450)
- **Muscular Dystrophies** (C16.320.577)
  - **Muscular Dystrophies, Limb-Girdle** (C16.320.577.280)
  - **Muscular Dystrophy, Duchenne** (C16.320.577.300)
  - **Muscular Dystrophy, Facioscapulohumeral** (C16.320.577.400)
- **Osteogenesis Imperfecta** (C16.320.737)
- Skin Diseases, Genetic (C16.320.850)
  - **Dermatitis, Atopic** (C16.320.850.210)
  - ~~**Epidermolysis Bullosa**~~ (C16.320.850.275)
    - ~~**Epidermolysis Bullosa Dystrophica**~~ (C16.320.850.275.160)
  - ~~Ichthyosiform Erythroderma, Congenital~~ (C16.320.850.400)
    - ~~**Netherton Syndrome**~~ (C16.320.850.400.705)
  - ~~**Netherton Syndrome**~~ (C16.320.850.673)
- Infant, Newborn, Diseases (C16.614)
  - ~~Ichthyosis~~ (C16.614.492)
    - ~~Ichthyosiform Erythroderma, Congenital~~ (C16.614.492.400)
      - ~~**Netherton Syndrome**~~ (C16.614.492.400.705)
  - Infant, Premature, Diseases (C16.614.521)
    - **Bronchopulmonary Dysplasia** (C16.614.521.125)
    - ~~**Respiratory Distress Syndrome, Newborn**~~ (C16.614.521.563)
- Skin and Connective Tissue Diseases (C17)
  - Connective Tissue Diseases (C17.300)
    - **Cartilage Diseases** (C17.300.182)
      - **Osteochondritis** (C17.300.182.520)
    - Collagen Diseases (C17.300.200)
      - ~~**Epidermolysis Bullosa Dystrophica**~~ (C17.300.200.367)
      - **Keloid** (C17.300.200.425)
      - **Osteogenesis Imperfecta** (C17.300.200.540)
    - **Lupus Erythematosus, Systemic** (C17.300.480)
      - **Lupus Nephritis** (C17.300.480.680)
    - **Penile Induration** (C17.300.715)
    - **Rheumatic Diseases** (C17.300.775)
      - **Arthritis, Rheumatoid** (C17.300.775.099)
        - **Sjogren's Syndrome** (C17.300.775.099.774)
    - **Scleroderma, Systemic** (C17.300.799)
      - **Scleroderma, Diffuse** (C17.300.799.602)
  - **Skin Diseases** (C17.800)
    - Breast Diseases (C17.800.090)
      - Breast Neoplasms (C17.800.090.500)
        - **Carcinoma, Ductal, Breast** (C17.800.090.500.390)
    - **Dermatitis** (C17.800.174)
      - **Dermatitis, Atopic** (C17.800.174.193)
    - ~~Foot Diseases~~ (C17.800.321)
      - ~~**Foot Ulcer**~~ (C17.800.321.250)
    - ~~Keratosis~~ (C17.800.428)
      - ~~Ichthyosis~~ (C17.800.428.333)
        - ~~Ichthyosiform Erythroderma, Congenital~~ (C17.800.428.333.250)
          - ~~**Netherton Syndrome**~~ (C17.800.428.333.250.705)
    - ~~**Scleroderma, Systemic**~~ (C17.800.784)
      - ~~**Scleroderma, Diffuse**~~ (C17.800.784.602)
    - Skin Abnormalities (C17.800.804)
      - **Epidermolysis Bullosa** (C17.800.804.493)
        - **Epidermolysis Bullosa Dystrophica** (C17.800.804.493.160)
      - Ichthyosis (C17.800.804.512)
        - Ichthyosiform Erythroderma, Congenital (C17.800.804.512.400)
          - **Netherton Syndrome** (C17.800.804.512.400.705)
    - ~~Skin Diseases, Eczematous~~ (C17.800.815)
      - ~~**Dermatitis, Atopic**~~ (C17.800.815.193)
    - ~~Skin Diseases, Genetic~~ (C17.800.827)
      - ~~**Dermatitis, Atopic**~~ (C17.800.827.210)
      - ~~**Epidermolysis Bullosa**~~ (C17.800.827.275)
        - ~~**Epidermolysis Bullosa Dystrophica**~~ (C17.800.827.275.160)
      - ~~Ichthyosiform Erythroderma, Congenital~~ (C17.800.827.400)
        - ~~**Netherton Syndrome**~~ (C17.800.827.400.705)
      - ~~**Netherton Syndrome**~~ (C17.800.827.655)
    - Skin Diseases, Metabolic (C17.800.849)
      - **Lipodystrophy** (C17.800.849.391)
    - Skin Diseases, Vascular (C17.800.862)
      - **Behcet Syndrome** (C17.800.862.150)
    - ~~Skin Diseases, Vesiculobullous~~ (C17.800.865)
      - ~~**Epidermolysis Bullosa**~~ (C17.800.865.410)


Epidermolysis Bullosa Dystrophica (C17.800.865.410.160)
Skin Ulcer (C17.800.893)
Leg Ulcer (C17.800.893.592)
Foot Ulcer (C17.800.893.592.450)
Diabetic Foot (C17.800.893.592.450.200)
Varicose Ulcer (C17.800.893.592.730)
Pressure Ulcer (C17.800.893.665)
Sweat Gland Diseases (C17.800.946)
Nutritional and Metabolic Diseases (C18)
Metabolic Diseases (C18.452)
Brain Diseases, Metabolic (C18.452.132)
Brain Diseases, Metabolic, Inborn (C18.452.132.100)
Hereditary Central Nervous System Demyelinating Diseases (C18.452.132.100.362)
Adrenoleukodystrophy (C18.452.132.100.362.250)
Pelizaeus-Merzbacher Disease (C18.452.132.100.362.775)
Peroxisomal Disorders (C18.452.132.100.680)
Adrenoleukodystrophy (C18.452.132.100.680.100)
Urea Cycle Disorders, Inborn (C18.452.132.100.937)
DNA Repair-Deficiency Disorders (C18.452.284)
Fanconi Anemia (C18.452.284.280)
Glucose Metabolism Disorders (C18.452.394)
Diabetes Mellitus (C18.452.394.750)
Diabetes Mellitus, Type 1 (C18.452.394.750.124)
Diabetes Mellitus, Type 2 (C18.452.394.750.149)
Lipid Metabolism Disorders (C18.452.584)
Dyslipidemias (C18.452.584.500)
Hyperlipidemias (C18.452.584.500.500)
Hypercholesterolemia (C18.452.584.500.500.396)
Hyperlipoproteinemias (C18.452.584.500.500.644)
Hyperlipoproteinemia Type II (C18.452.584.500.500.644.475)
Lipodystrophy (C18.452.584.625)
Lipidoses (C18.452.584.687)
Neuronal Ceroid-Lipofuscinoses (C18.452.584.687.509)
Metabolism, Inborn Errors (C18.452.648)
Amino Acid Metabolism, Inborn Errors (C18.452.648.100)
Urea Cycle Disorders, Inborn (C18.452.648.100.940)
Brain Diseases, Metabolic, Inborn (C18.452.648.189)
Hereditary Central Nervous System Demyelinating Diseases (C18.452.648.189.362)
Adrenoleukodystrophy (C18.452.648.189.362.250)
Pelizaeus-Merzbacher Disease (C18.452.648.189.362.775)
Peroxisomal Disorders (C18.452.648.189.680)
Adrenoleukodystrophy (C18.452.648.189.680.100)
Urea Cycle Disorders, Inborn (C18.452.648.189.937)
Hyperbilirubinemia, Hereditary (C18.452.648.300)
Crigler-Najjar Syndrome (C18.452.648.300.281)
Lipid Metabolism, Inborn Errors (C18.452.648.398)
Hyperlipoproteinemia Type II (C18.452.648.398.481)
Lipidoses (C18.452.648.398.641)
Neuronal Ceroid-Lipofuscinoses (C18.452.648.398.641.509)
Peroxisomal Disorders (C18.452.648.663)
Adrenoleukodystrophy (C18.452.648.663.112)
Renal Tubular Transport, Inborn Errors (C18.452.648.861)
Fanconi Syndrome (C18.452.648.861.450)
Proteostasis Deficiencies (C18.452.845)
TDP-43 Proteinopathies (C18.452.845.800)
Amyotrophic Lateral Sclerosis (C18.452.845.800.050)
Skin Diseases, Metabolic (C18.452.880)
Lipodystrophy (C18.452.880.391)
Endocrine System Diseases (C19)
Adrenal Gland Diseases (C19.053)
Adrenal Insufficiency (C19.053.500)
Adrenoleukodystrophy (C19.053.500.270)
Diabetes Mellitus (C19.246)
Diabetes Complications (C19.246.099)
Diabetic Angiopathies (C19.246.099.500)
Diabetic Foot (C19.246.099.500.191)
Diabetic Retinopathy (C19.246.099.500.382)
Diabetic Nephropathies (C19.246.099.875)
Diabetic Neuropathies (C19.246.099.937)
Diabetic Foot (C19.246.099.937.250)
Diabetes Mellitus, Type 1 (C19.246.267)
Diabetes Mellitus, Type 2 (C19.246.300)
Gonadal Disorders (C19.391)
Disorders of Sex Development (C19.391.119)
Sex Chromosome Disorders of Sex Development (C19.391.119.795)
Klinefelter Syndrome (C19.391.119.795.500)
Hypogonadism (C19.391.482)
Klinefelter Syndrome (C19.391.482.629)
Ovarian Diseases (C19.391.630)
Ovarian Neoplasms (C19.391.630.705)
Carcinoma, Endometrioid (C19.391.630.705.331)
Primary Ovarian Insufficiency (C19.391.630.750)
Immune System Diseases (C20)
Autoimmune Diseases (C20.111)
Arthritis, Rheumatoid (C20.111.199)
Sjogren's Syndrome (C20.111.199.774)
Autoimmune Diseases of the Nervous System (C20.111.258)
Demyelinating Autoimmune Diseases, CNS (C20.111.258.250)
Multiple Sclerosis (C20.111.258.250.500)
Multiple Sclerosis, Chronic Progressive (C20.111.258.250.500.200)
Multiple Sclerosis, Relapsing-Remitting (C20.111.258.250.500.600)
Neuromyelitis Optica (C20.111.258.250.500.650)
Myelitis, Transverse (C20.111.258.250.550)

- Neuromyelitis Optica (C20.111.258.250.550.500)
  - Neuromyelitis Optica (C20.111.258.250.600)
- Diabetes Mellitus, Type 1 (C20.111.327)
- Hepatitis, Autoimmune (C20.111.567)
- Lupus Erythematosus, Systemic (C20.111.590)
  - Lupus Nephritis (C20.111.590.560)
- Graft vs Host Disease (C20.452)
- Hypersensitivity (C20.543)
  - Hypersensitivity, Immediate (C20.543.480)
    - Dermatitis, Atopic (C20.543.480.343)
- Immunologic Deficiency Syndromes (C20.673)
  - HIV Infections (C20.673.480)
    - Acquired Immunodeficiency Syndrome (C20.673.480.040)
- Pathological Conditions, Signs and Symptoms (C23)
  - Pathological Conditions, Anatomical (C23.300)
    - Atrophy (C23.300.070)
    - Constriction, Pathologic (C23.300.287)
    - Fistula (C23.300.575)
      - Digestive System Fistula (C23.300.575.185)
        - Intestinal Fistula (C23.300.575.185.550)
          - Rectal Fistula (C23.300.575.185.550.600)
            - Rectovaginal Fistula (C23.300.575.185.550.600.650)
      - Vaginal Fistula (C23.300.575.925)
        - Rectovaginal Fistula (C23.300.575.925.558)
    - Hernia (C23.300.707)
      - Intervertebral Disc Displacement (C23.300.707.952)
    - Leg Length Inequality (C23.300.808)
  - Pathologic Processes (C23.550)
    - Ascites (C23.550.081)
    - Delayed Graft Function (C23.550.277)
    - Disease (C23.550.288)
      - Syndrome (C23.550.288.500)
    - Emphysema (C23.550.325)
    - Fibrosis (C23.550.355)
      - Cicatrix (C23.550.355.274)
        - Keloid (C23.550.355.274.510)
    - Hemorrhage (C23.550.414)
      - Intracranial Hemorrhages (C23.550.414.913)
        - Cerebral Hemorrhage (C23.550.414.913.100)
    - Inflammation (C23.550.470)
      - Foreign-Body Reaction (C23.550.470.251)
      - Systemic Inflammatory Response Syndrome (C23.550.470.790)
        - Sepsis (C23.550.470.790.500)
          - Shock, Septic (C23.550.470.790.500.800)
    - Ischemia (C23.550.513)
      - Infarction (C23.550.513.355)
    - Necrosis (C23.550.717)
      - Dental Pulp Necrosis (C23.550.717.182)
      - Gangrene (C23.550.717.427)
      - Infarction (C23.550.717.489)
    - Osteonecrosis (C23.550.717.732)
      - Femur Head Necrosis (C23.550.717.732.368)
    - Nerve Degeneration (C23.550.737)
    - Sclerosis (C23.550.823)
    - Shock (C23.550.835)
      - Multiple Organ Failure (C23.550.835.525)
      - Systemic Inflammatory Response Syndrome (C23.550.835.900)
        - Shock, Septic (C23.550.835.900.712)
    - Ulcer (C23.550.891)
  - Signs and Symptoms (C23.888)
    - Intermittent Claudication (C23.888.531)
    - Neurologic Manifestations (C23.888.592)
      - Dyskinesias (C23.888.592.350)
        - Ataxia (C23.888.592.350.090)
          - Cerebellar Ataxia (C23.888.592.350.090.200)
      - Neurobehavioral Manifestations (C23.888.592.604)
        - Intellectual Disability (C23.888.592.604.646)
      - Pain (C23.888.592.612)
        - Back Pain (C23.888.592.612.107)
          - Low Back Pain (C23.888.592.612.107.400)
      - Paralysis (C23.888.592.636)
        - Ophthalmoplegia (C23.888.592.636.447)
          - Supranuclear Palsy, Progressive (C23.888.592.636.447.690)
      - Sensation Disorders (C23.888.592.763)
        - Hearing Disorders (C23.888.592.763.393)
          - Hearing Loss (C23.888.592.763.393.341)
            - Deafness (C23.888.592.763.393.341.186)
            - Hearing Loss, Sensorineural (C23.888.592.763.393.341.887)
    - Pain (C23.888.646)
      - Back Pain (C23.888.646.172)
        - Low Back Pain (C23.888.646.172.500)
      - Chest Pain (C23.888.646.215)
        - Angina Pectoris (C23.888.646.215.500)
          - Angina, Stable (C23.888.646.215.500.575)
    - Urological Manifestations (C23.888.942)
      - Lower Urinary Tract Symptoms (C23.888.942.343)
        - Urinary Incontinence (C23.888.942.343.800)
          - Urinary Incontinence, Stress (C23.888.942.343.800.500)
- Occupational Diseases (C24)
  - Pneumoconiosis (C24.800)
    - Silicosis (C24.800.834)
- Chemically-Induced Disorders (C25)
  - Poisoning (C25.723)
  - Substance-Related Disorders (C25.775)
    - Alcohol-Related Disorders (C25.775.100)
      - Alcohol-Induced Disorders (C25.775.100.087)
        - Liver Diseases, Alcoholic (C25.775.100.087.645)
          - Liver Cirrhosis, Alcoholic (C25.775.100.087.645.550)

Supplementary File 1.

Refinement of the resulting MeSH terms before phylogenetic-like tree visualization.

This file provides the final MeSH structure used. Bold: MeSH terms that were selected. Underlined: proximal branches of selected MeSH terms. Crossed out: branches that were removed for phylogenetic-like tree visualization.

Figure 1

Cette représentation est un circos, ou connectogramme. Il s'agit d'une représentation visuelle permettant d'analyser la complexité d'un tableau à double entrée. Chaque ligne et chaque colonne est codée selon une couleur spécifique. Par exemple, les années sont codées selon un niveau de gris (légende des gris à gauche de la figure), alors que les différents champs d'application de la branche maladie du MeSH sont codés par couleur (légende couleur à droite de la figure).

Nous pouvons donc avoir deux informations :

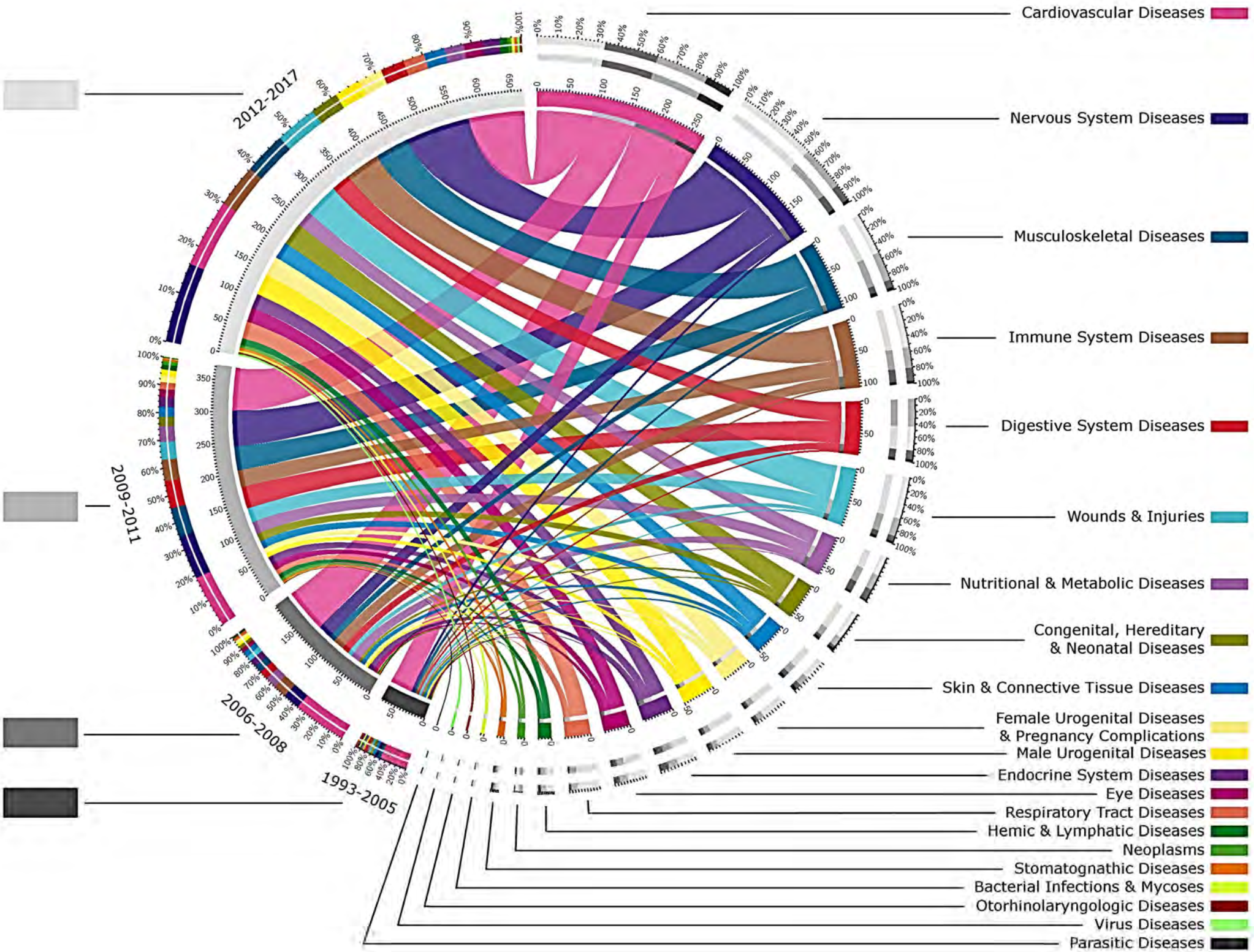
- La contribution des différents champs d'application pour chaque catégorie d'années : à gauche de la figure, pour chaque catégorie d'années, il existe un segment pour chaque couleur des champs d'application contributifs. La partie externe du diagramme montre la proportion de la contribution du champ à l'année. La partie interne permet de mesurer le nombre d'études.
- Inversement, pour chaque champ d'application à droite de la figure, il existe un segment pour chaque niveau de gris des années contributives. La partie externe du diagramme montre la proportion de la contribution de l'année au champ. La partie interne permet de mesurer le nombre d'études.

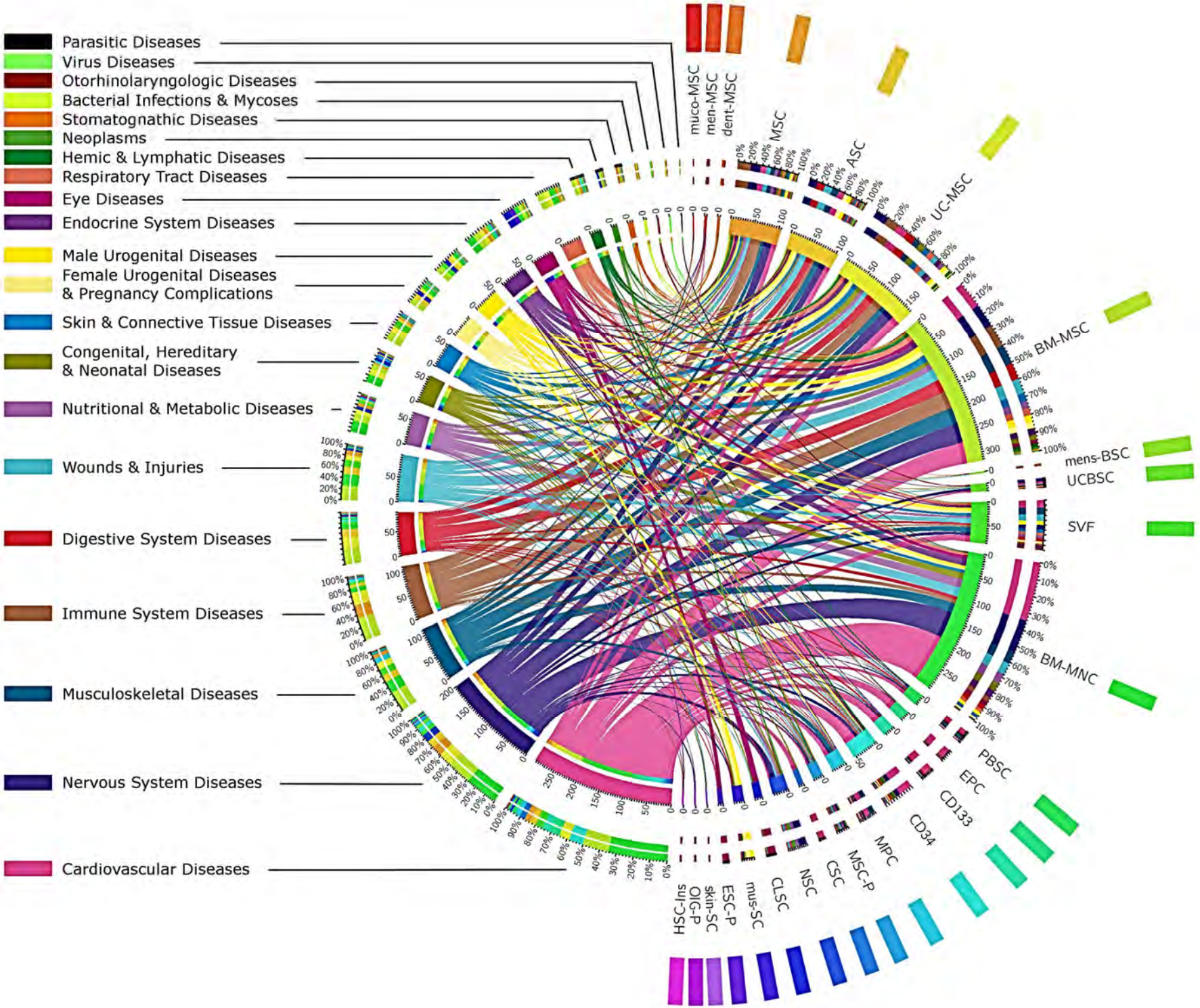
Ainsi, dans le champ du cardiovasculaire (partie gauche), 70 études ont commencé en 2006-2008 (comme indiqué sur la partie interne du diagramme), ce qui représente 36% des champs en 2006-2008 (comme indiqué à gauche sur la partie externe du diagramme). Inversement 25% des essais cliniques sur le cardiovasculaire (partie droite) ont commencé en 2006-2008 (indiqué à droite sur la partie externe du circos).

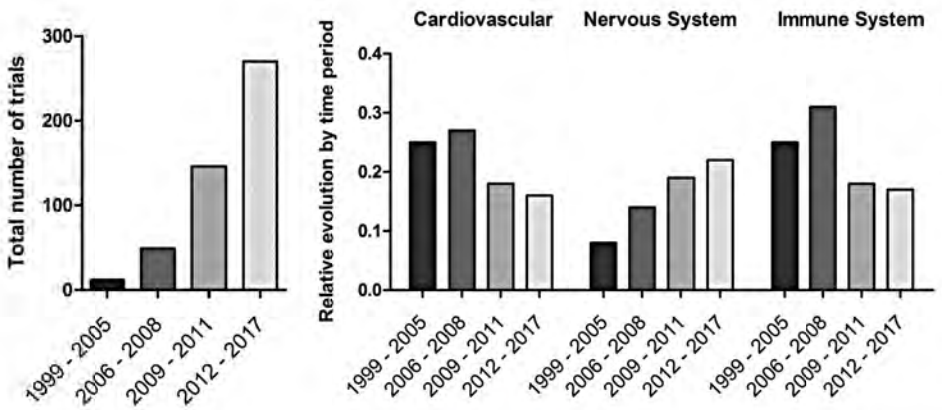
Même s'il pourrait être dit à partir de ce graphique que 36% des études en 2006-2008 sont en cardio-vasculaire, cette interprétation est fausse. En effet, le MeSH est redondant, c'est-à-dire qu'un même mot clef MeSH peut se retrouver dans différents champs d'application. C'est pour cela que l'interprétation la plus juste est que le champ cardio-vasculaire représente 36% des champs d'application en 2006-2008. Dans la figure 3, nous avons rajouté un histogramme afin de montrer le nombre précis d'études par année.

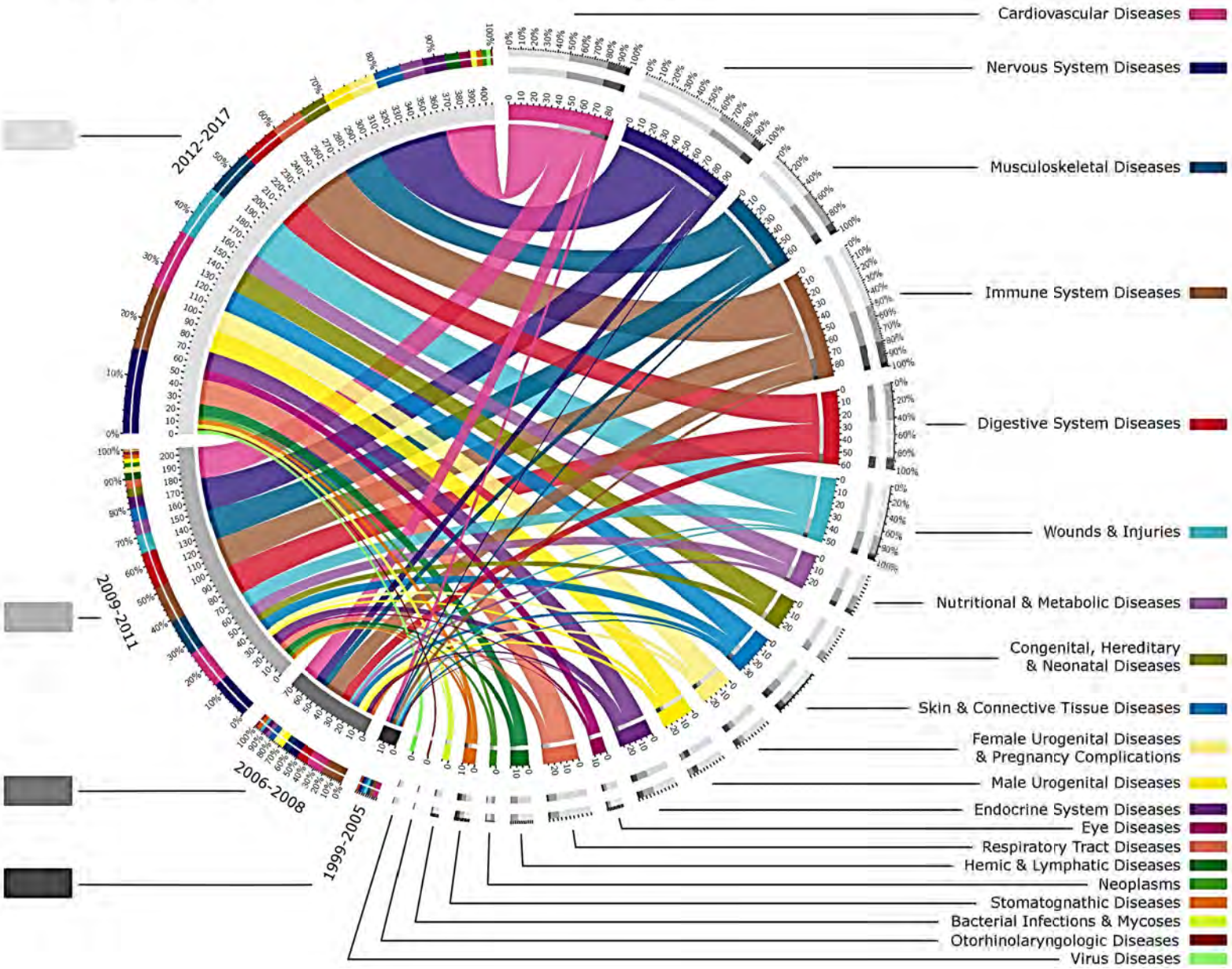
L'interprétation des Figures 2, 3 et 4 pour cet article est identique.

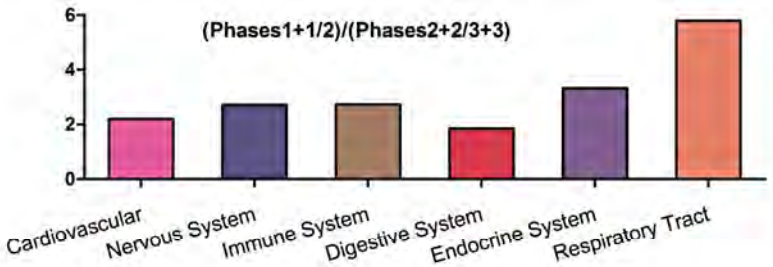
L'interprétation des Figures 1 et 4 dans Journal of Clinical Periodontology est identique.

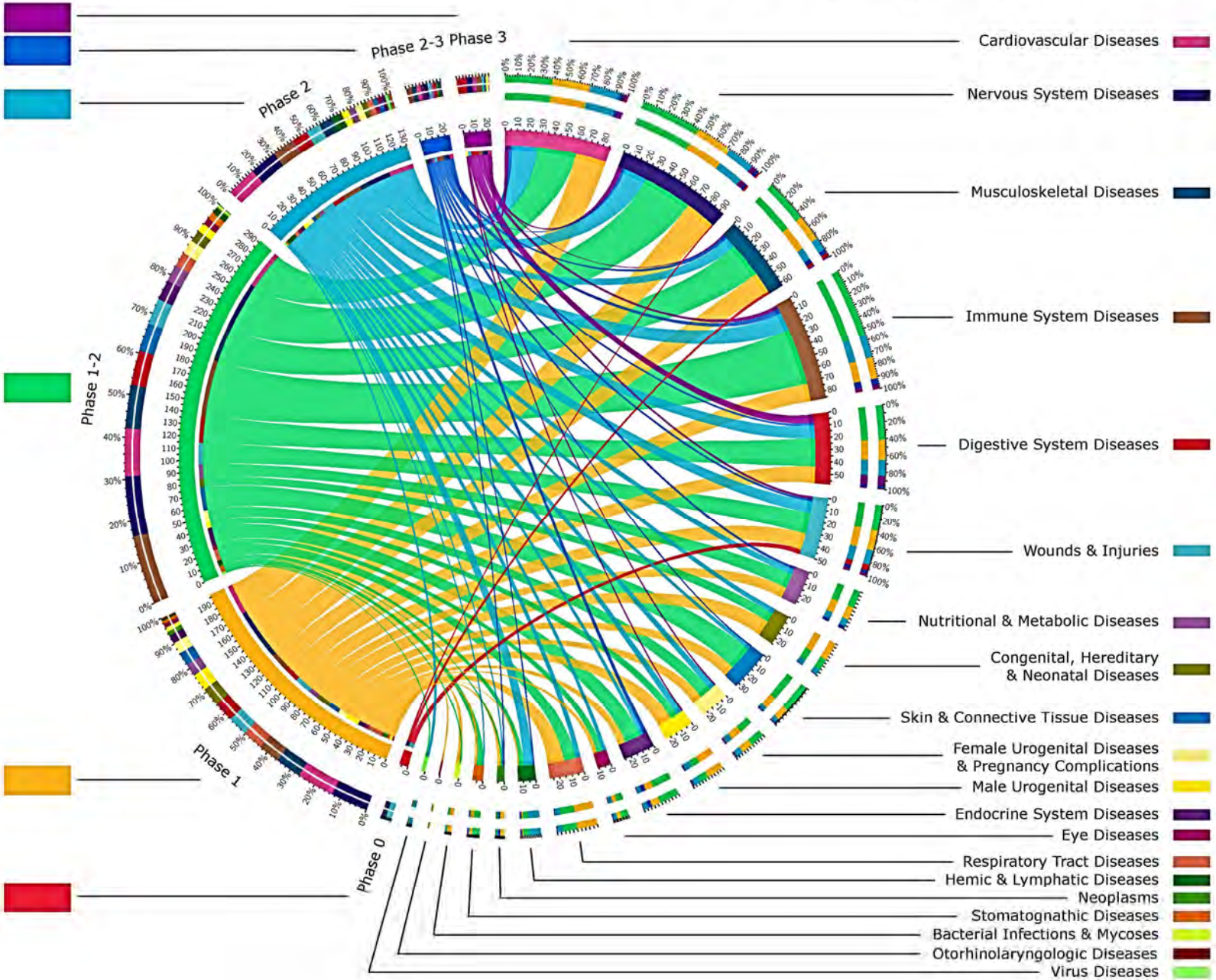

2012-2017
2009-2011
2006-2008
1993-2005
Cardiovascular Diseases
Nervous System Diseases
Musculoskeletal Diseases
Immune System Diseases
Digestive System Diseases
Wounds & Injuries
Nutritional & Metabolic Diseases
Congenital, Hereditary & Neonatal Diseases
Skin & Connective Tissue Diseases
Female Urogenital Diseases & Pregnancy Complications
Male Urogenital Diseases
Endocrine System Diseases
Eye Diseases
Respiratory Tract Diseases
Hemic & Lymphatic Diseases
Neoplasms
Stomatognathic Diseases
Bacterial Infections & Mycoses
Otorhinolaryngologic Diseases
Virus Diseases
Parasitic Diseases


Parasitic Diseases
Virus Diseases
Otorhinolaryngologic Diseases
Bacterial Infections & Mycoses
Stomatognathic Diseases
Neoplasms
Hemic & Lymphatic Diseases
Respiratory Tract Diseases
Eye Diseases
Endocrine System Diseases
Male Urogenital Diseases
Female Urogenital Diseases & Pregnancy Complications
Skin & Connective Tissue Diseases
Congenital, Hereditary & Neonatal Diseases
Nutritional & Metabolic Diseases
Wounds & Injuries
Digestive System Diseases
Immune System Diseases
Musculoskeletal Diseases
Nervous System Diseases
Cardiovascular Diseases
muco-MSC
men-MSC
dent-MSC
MSC
ASC
UC-MSC
BM-MSC
mens-BSC
UCBSC
SVF
BM-MNC
PBSC
EPC
CD133
CD34
MPC
MSC-P
CSC
NSC
CLSC
mus-SC
skin-SC
ESC-P
OLG-P
HSC-Ins


Total number of trials
300
200
100
0
1999 - 2005
2006 - 2008
2009 - 2011
2012 - 2017
Relative evolution by time period
Cardiovascular
Nervous System
Immune System
0.4
0.3
0.2
0.1
0.0
1999 - 2005
2006 - 2008
2009 - 2011
2012 - 2017


2012-2017
2009-2011
2006-2008
1999-2005
Cardiovascular Diseases
Nervous System Diseases
Musculoskeletal Diseases
Immune System Diseases
Digestive System Diseases
Wounds & Injuries
Nutritional & Metabolic Diseases
Congenital, Hereditary & Neonatal Diseases
Skin & Connective Tissue Diseases
Female Urogenital Diseases & Pregnancy Complications
Male Urogenital Diseases
Endocrine System Diseases
Eye Diseases
Respiratory Tract Diseases
Hemic & Lymphatic Diseases
Neoplasms
Stomatognathic Diseases
Bacterial Infections & Mycoses
Otorhinolaryngologic Diseases
Virus Diseases


(Phases1+1/2)/(Phases2+2/3+3)
6
4
2
0
Cardiovascular
Nervous System
Immune System
Digestive System
Endocrine System
Respiratory Tract


Phase 2-3 Phase 3
Phase 2
Phase 1-2
Phase 1
Phase 0
Cardiovascular Diseases
Nervous System Diseases
Musculoskeletal Diseases
Immune System Diseases
Digestive System Diseases
Wounds & Injuries
Nutritional & Metabolic Diseases
Congenital, Hereditary & Neonatal Diseases
Skin & Connective Tissue Diseases
Female Urogenital Diseases & Pregnancy Complications
Male Urogenital Diseases
Endocrine System Diseases
Eye Diseases
Respiratory Tract Diseases
Hemic & Lymphatic Diseases
Neoplasms
Stomatognathic Diseases
Bacterial Infections & Mycoses
Otorhinolaryngologic Diseases
Virus Diseases

I.3.2 Principaux éléments de discussion et perspectives

Un champ clinique dominé par les cellules souches adultes

Notre analyse révèle une frilosité quant à l'utilisation de cellules embryonnaires et fœtales ; le champ est quasiment exclusivement réduit aux hESCs-RPE, des cellules différenciées à partir d'ESCs et implantées en faible nombre dans le site immunoprilégié qu'est l'œil (40). Malgré l'augmentation des financements pour les ESCs dans les années 2000, la difficulté de légiférer (41), l'opinion défavorable de la population (42), les considérations éthiques, religieuses, pourraient expliquer la faiblesse de ce champ. Ceci pourrait également traduire l'explosion des études et des champs d'applications couverts par les cellules souches adultes à partir de 2005. Existe-t-il une relation entre le cadre législatif sur les ESCs et la production d'essais cliniques sur les cellules souches dans un pays ?

Une absence de spécificité cellulaire vis-à-vis d'une pathologie

Une fois que l'on retire les études en relation avec l'utilisation des cellules souches hématopoïétiques après chimiothérapie ou radiothérapie (33), nous nous rendons compte qu'il n'existe pas de consensus sur l'utilisation spécifique de types de cellules souches vis-à-vis de pathologies ciblées. Leur utilisation semble donc se révéler plus de l'initiative personnelle et de facteurs techniques, que d'un rationnel physiopathologique.

Ces thérapeutiques semblent traiter toutes les pathologies de par leur pluri/multi potentialité, et leur activité immuno-régulatrice/immuno-suppressive (43). Il s'agit du rêve de l'industrie de détenir un produit « universel ». Le produit thérapeutique est très mal défini, pluri/multipotent, hétérogène non purifié ou purifié, cellules cultivées ou sélectionnées, autologue ou allogène ; et ceci sans réelle étude comparative. Nous sommes bien éloignés de la vision d'un produit pour une cible thérapeutique (44). De plus, les données précliniques sont encore maigres. Peu d'études comparent encore les différentes sources de cellules entre elles (45, 46).

Nous suggérons donc une veille bibliographique sur la publication des essais cliniques afin que de futures revues systématiques et méta-analyses puissent tenter de faire ressortir le bénéfice de certaines sources de CSMs sur certaines pathologies. Les études précliniques, essentiellement animales, sont également indispensables afin de comparer scrupuleusement l'efficacité de différentes sources de CSMs quant à l'objectif de régénération à atteindre. Des allers-retours entre préclinique et clinique sont donc nécessaires.

L'évolution vers les cellules cultivées

La fraction cellulaire hétérogène (produit autologue) est accessible au lit du malade en quelques heures et de manière peu couteuse ; ceci peut expliquer la maturité plus avancée de ces études comparativement aux utilisations de cellules cultivées. Pourtant, nous avons mis en évidence un net recul de cette fraction hétérogène par rapport aux cellules cultivées ces dernières années.

Cet effet de déclin pourrait être lié à des différences importantes et des contradictions dans de nombreuses études du champ cardiovasculaire (47, 48), malgré les résultats de méta-analyses Cochrane (49, 50). Il est en effet possible que des raisons techniques (la manière de gérer le contrôle *sham*) puissent être à l'origine d'une surestimation de l'effet de ces fractions cellulaires brutes (51). Une attention toute particulière devra être portée à la gestion du contrôle dans les essais cliniques ; le groupe placebo devra subir comme le groupe traitement un prélèvement de tissu pour cultiver les cellules. Les BM-MSCs ont donc été plus récemment explorées (52), et de futures méta-analyses évalueront leurs effets. De plus, plusieurs études précliniques et cliniques ont démontré la supériorité des BM-MSC par rapport aux BMMNCs pour les pathologies cardiaques ischémiques ou les ischémies des membres inférieurs et ulcères des pieds diabétiques (45, 53).

Un mécanisme régénératif de l'utilisation des CSM encore mal connu

Concernant plus spécifiquement les CSMs, seuls trois grands types cellulaires intéressent la recherche, les BM-MSCs, ASCs et UC-MSCs (« Umbilical Cord derived Mesenchymal Stromal Cells ») même si ces cellules peuvent être potentiellement isolées de n'importe quel organe (54). Les BM-MSCs bénéficient du recul clinique le plus important (55), les ASCs sont isolables en grand nombre avec une faible morbidité à partir de prélèvements adipeux réalisés sous anesthésie locale (56-58) et les UC-MSCs peuvent être facilement stockées dans des banques cellulaires tout en étant qualifiées de plus « plastiques » de par leur origine (59). S'il parait logique d'implanter des ASCs pour traiter des lipodystrophies, des BM-MSCs pour traiter des lésions osseuses, des utilisations de CSMs d'origine ectopique au tissu cible (pour la maladie du greffon contre l'hôte, la maladie de Crohn, les pathologies neurologiques), sont beaucoup plus surprenantes. En effet, ces utilisations entrent en contradiction avec les possibles propriétés biologiques des CSMs que leur environnement initial leur confère (60). Mais ceci serait compensé par le rôle des CSMs en tant que cellules de soutien, trophiques, aspécifiques, tissu/organe indépendant (36).

L'augmentation de l'utilisation des cellules allogéniques

Les CSMs sont considérées comme à faible risque immunogénique (faible expression de HLA). Pourtant, l'expression de ces marqueurs augmente avec la mise en culture (36), et des cas de rejet ont été documentés (36, 61, 62). En contrepartie, l'élimination quasi-totale des CSMs administrées dans un contexte allogénique garantit une présence uniquement transitoire des cellules dans l'organisme hôte, qui, si elle suffit à gérer et résoudre à long terme une situation tissulaire compromise, peut de plus être considérée comme un élément de sécurité favorable pour le patient.

La possibilité d'obtenir en grand nombre des CSMs, à partir de donneurs indemnes de pathologies systémiques rend l'allogénique particulièrement attractif pour les industriels. Afin d'essayer de mieux cerner ces éléments, je codirige actuellement une thèse d'Etat de Docteur en Chirurgie Dentaire dont l'objectif est de déterminer quels sont les facteurs du donneur impliqués dans la modification des caractéristiques des ASCs *in-vivo* et *in-vitro* (e.g. potentiel de différenciation et de prolifération, potentiel régénératif après greffe dans des modèles animaux) dans un objectif d'anticipation de thérapie cellulaire.

I.4 Structuration de la recherche sur les CSMs

I.4.1 Article 2: "Mesenchymal stem cell based therapy: a network analysis of the clinical trials"

Le travail présenté dans ce chapitre fait l'objet d'un article en cours de finalisation pour soumission au journal Cytotherapy.

I.4.2 Introduction

La constitution de cette agrégation de données provenant de CTD, telle que décrite plus haut, permet d'envisager de nouveaux projets, une nouvelle réflexion. Notre analyse a fait émerger des questions précises quant au rôle moteur de certains pays, de certaines villes, leur interaction, leur capacité à travailler en groupe, en réseau. Le rôle des sponsors est également un point délicat : un financement industriel permet-il d'avancer plus vite dans les travaux scientifiques ? Influe-t-il sur la manière dont les chercheurs travaillent en commun ? Quel rôle jouent les pays asiatiques dans l'échiquier mondial ? Quel est l'influence de la politique scientifique des pays en terme de régulation pour les cellules souches, sur la capacité des chercheurs à mener des études ? Certains pays, par un manque de législation, laissent des cliniques privées se développer où des traitements avec des CSMs sont proposés aux patients. Ces pays sont-ils plus avancés que les autres au niveau de l'élaboration des essais cliniques ?

Voici donc un échantillon de questions que nous nous posons et auxquelles nous allons tenter de répondre à travers une nouvelle analyse de notre échantillon constitué (tel que décrit plus haut) de la base CTD. Nous baserons en partie notre analyse sur la théorie de l'analyse sociale des réseaux, et la théorie des graphes, empruntées aux sciences sociales et surtout utilisées en biologie dans l'étude de l'expression génique (63, 64).

I.4.3 Matériels et méthodes

Notre unité statistique est la ville, que nous assimilons à un centre de recherche (avec la limite que s'il existait différents centres ou services utilisant les CSMs au sein d'une même ville, ils étaient considérés comme une seule et même entité).

Pour chaque essai clinique de CTD utilisant des CSMs, nous avons extrait leurs villes/centres. S'il existe plusieurs centres, nous les relions ensemble en forme de couronne, par ordre aléatoire. Au final, l'ensemble des études analysées permet de représenter un réseau.

Pour chaque ville, nous avons vérifié son unicité. En effet, certaines villes peuvent posséder plusieurs dénominations (comme Beijin/Pékin), ou une même dénomination peut en fait cacher des villes différentes (comme la ville de Springfield retrouvée dans plusieurs états aux Etats-Unis). Nous avons ensuite évalué la population de chaque ville selon le dernier recensement disponible grâce au site http://www.citypopulation.de/. Enfin nous avons noté de 1 à 4 chaque ville selon le degré de restriction du pays quant à l'utilisation des ESCs (1 : restrictif, 2 : moins restrictif, 3 : non restrictif, 4 : pas de politique mise en place) et grâce à la carte ci-dessous (Figure I-5)(65).

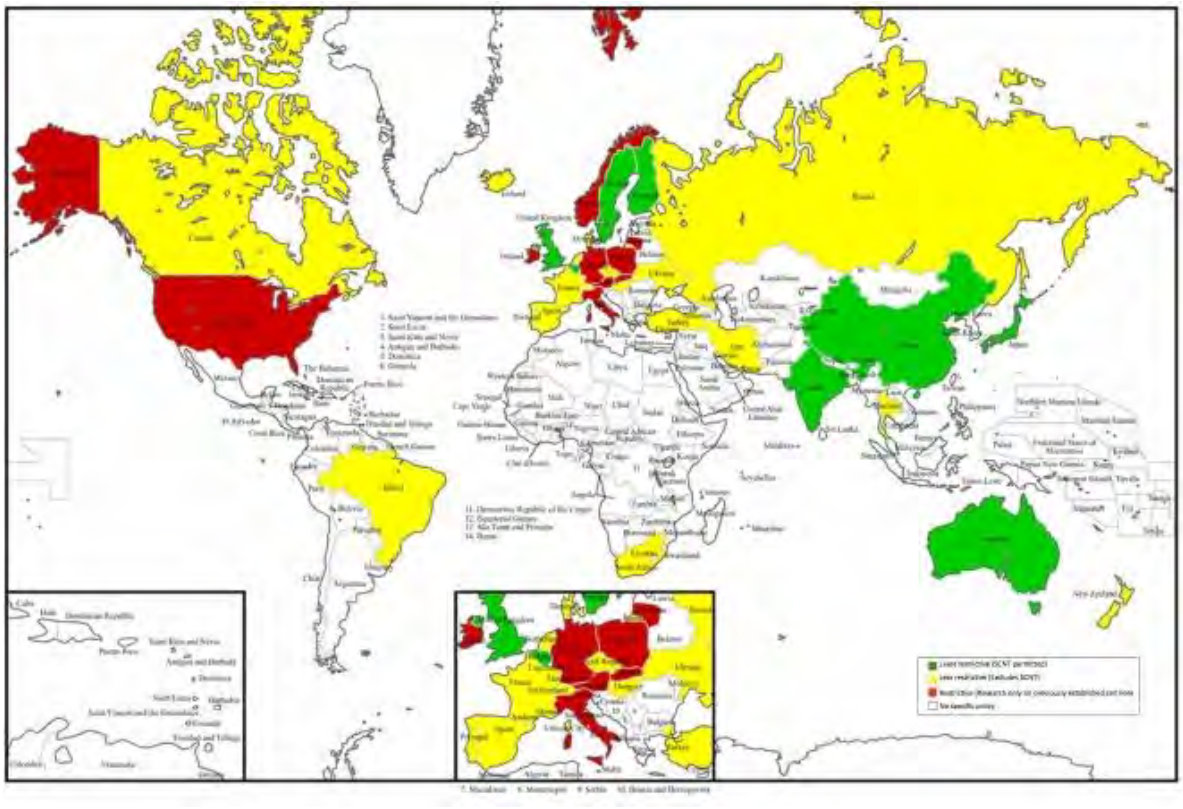


Figure I-5 : Mappemonde représentant la permissivité des pays quant à la législation vis-à-vis des ESCs. Vert, les moins restrictives, jaune moins restrictives et rouge très restrictives. Carte empruntée à (65).

La représentation graphique utilisée est une représentation de réseaux, créée en utilisant le logiciel Cytoscape 3.2.1 à partir des données de CTD exportées par un script spécifique de notre programme.

Paramètre réseau/nœud	**Description**
Nombre moyen de voisins	Nombre moyen de connexions entre les centres.
Densité du réseau	La densité (entre 0 et 1) est le nombre de connexions réelles entre les villes, divisé par le nombre de connexions possibles. Une densité plus élevée indique une plus grande interaction entre les villes dans le processus de participation à des essais cliniques.
Centralisation du réseau	Ce score (entre 0 et 1) indique le degré d'asymétrie dans la répartition des connexions dans le réseau. Un score élevé de centralisation indique que certains centres ont beaucoup plus de connexions que d'autres.
Betweenness centrality	Ce paramètre indique le rôle d'intermédiaire d'un centre dans le réseau. Son augmentation reflète le contrôle de la ville sur la communication entre d'autres villes.
Closeness centrality	Ce paramètre reflète la proximité entre les centres.
Hétérogénéité du réseau	Il s'agit d'un score de dispersion du degré des nœuds. Une forte hétérogénéité reflète une grande disparité entre les villes.
Coefficient de clustering	Il s'agit de la probabilité que deux villes qui sont connectées à la même ville, soit elles-mêmes reliées. Il mesure la recherche qui a été effectuée dans des groupes en collaboration.
Connectivité des voisins	Nombre moyen de voisins des voisins.
Score de phase	Score entre 0 et 1 représentant la maturité des études par rapport à leur maturité maximale possible ; Un score de 0 indique que la totalité des études sont de phase 1 alors qu'un score de 1 indique que la totalité des études sont de phase 3.
Score industriel	Proportion d'études possédant un financement majoritairement industriel. Nous nous basons sur l'algorithme défini par Califf et al. (66).
Etudes terminées	Proportion des études qui sont terminées.
Degré	Nombre d'études partagées avec d'autres villes.
Taille du nœud	Nombre d'études auxquelles participe chaque centre.
Partenaires multiples	Proportion de villes partenaires avec lesquelles au moins deux études sont partagées.

Table I-1 : les différentes propriétés des réseaux.

Différentes propriétés de ces réseaux peuvent donc ainsi être analysées (Table I-1).

I.4.4 Résultats et discussion

Concernant les CSMs

Après avoir importé les données dans Cytoscape, nous avons reconstruit le réseau en associant certaines propriétés graphiques aux propriétés de réseau telles que décrites plus haut (Table I-1). Nous avons un lien par étude dont la largeur est proportionnelle au nombre de patients dans l'essai clinique ; les nœuds isolés n'ont pas de lien. Chaque ville (nœud) est représentée par un cercle dont le diamètre est proportionnel au nombre d'études auxquelles participe le centre. Le niveau de gris interne indique le score de phase (noir, phase 0 ; blanc, phase 3). La couleur externe représente le continent (rouge pour l'Asie, vert pour l'Amérique du Nord, bleu pour l'Europe, marron pour l'Océanie, jaune pour l'Afrique, violet pour l'Amérique du Sud). Il existe une force de type ressort entre les nœuds, c'est-à-dire que les nœuds ont tendance à se repousser ; l'état de ce réseau est donc un état d'équilibre lié à ces forces (Figure I-6).

Le premier élément frappant est la répartition des villes au sein du réseau : les villes d'Amérique du Nord travaillent ensemble en quasi exclusivité, tout comme les villes d'Europe et d'Asie respectivement entre elles. Seules les villes d'Océanie semblent jouer un rôle de connecteur entre continent Américain et Européen (Figure I-7). Les villes asiatiques et d'Amérique du Sud sont plus isolées que le reste des villes (44.6% et 66.7%) alors que les villes d'Océanie sont connectées à 100% (Table I-2).

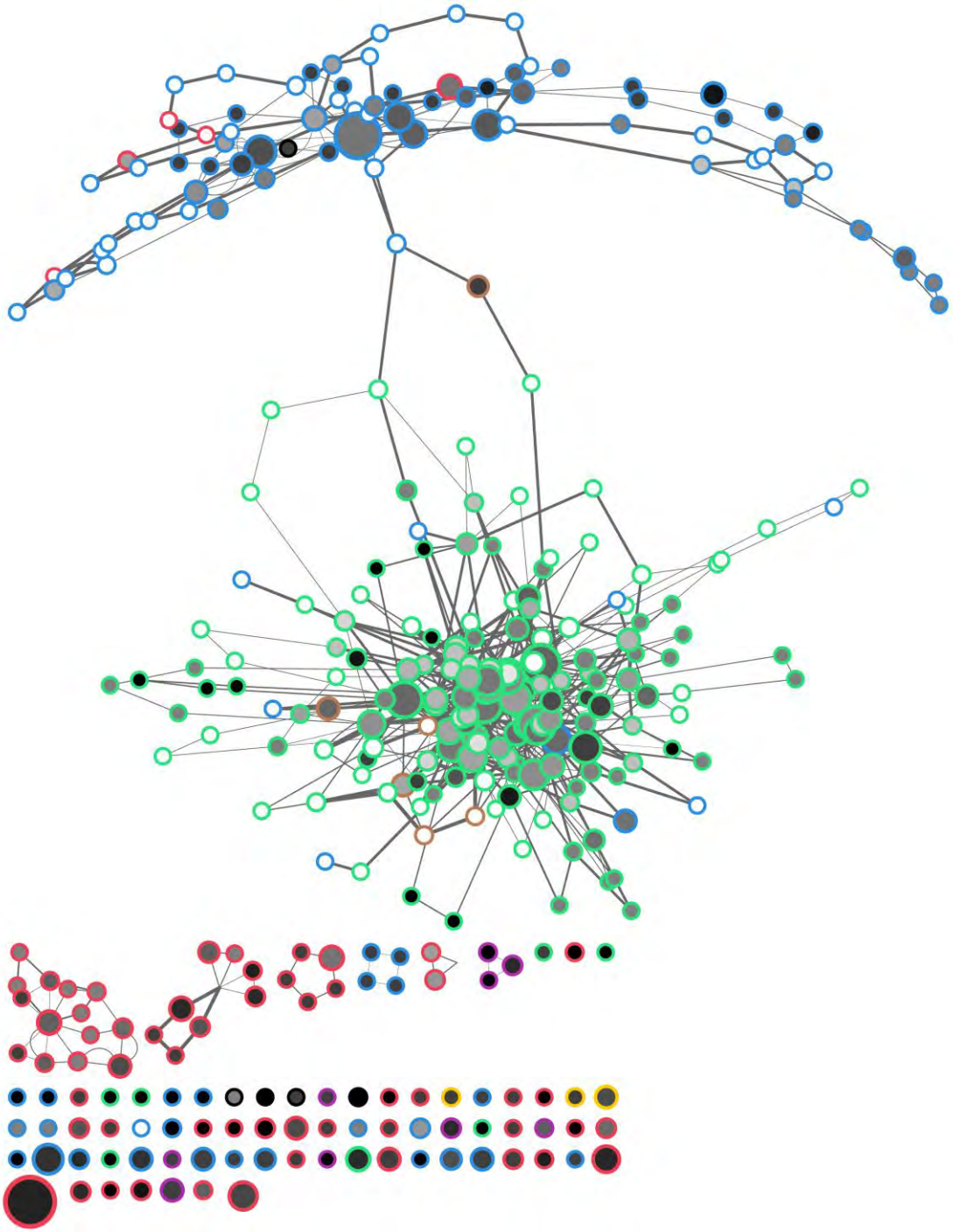


Figure I-6 : Réseau global des essais cliniques utilisant des MSCs. Réseau avec un lien par étude dont la largeur est proportionnelle au nombre de patients dans l'essai clinique. Chaque ville (nœud) est représentée par un cercle dont le diamètre est proportionnel au nombre d'études auquel participe le centre. Le niveau de gris interne indique le score de phase (noir, phase 0 ; blanc, phase 3). La couleur externe représente le continent (rouge Asie, vert Amérique du Nord, bleu Europe, marron Océanie, jaune Afrique, violet Amérique du Sud). Il existe une force de type ressort entre les nœuds.

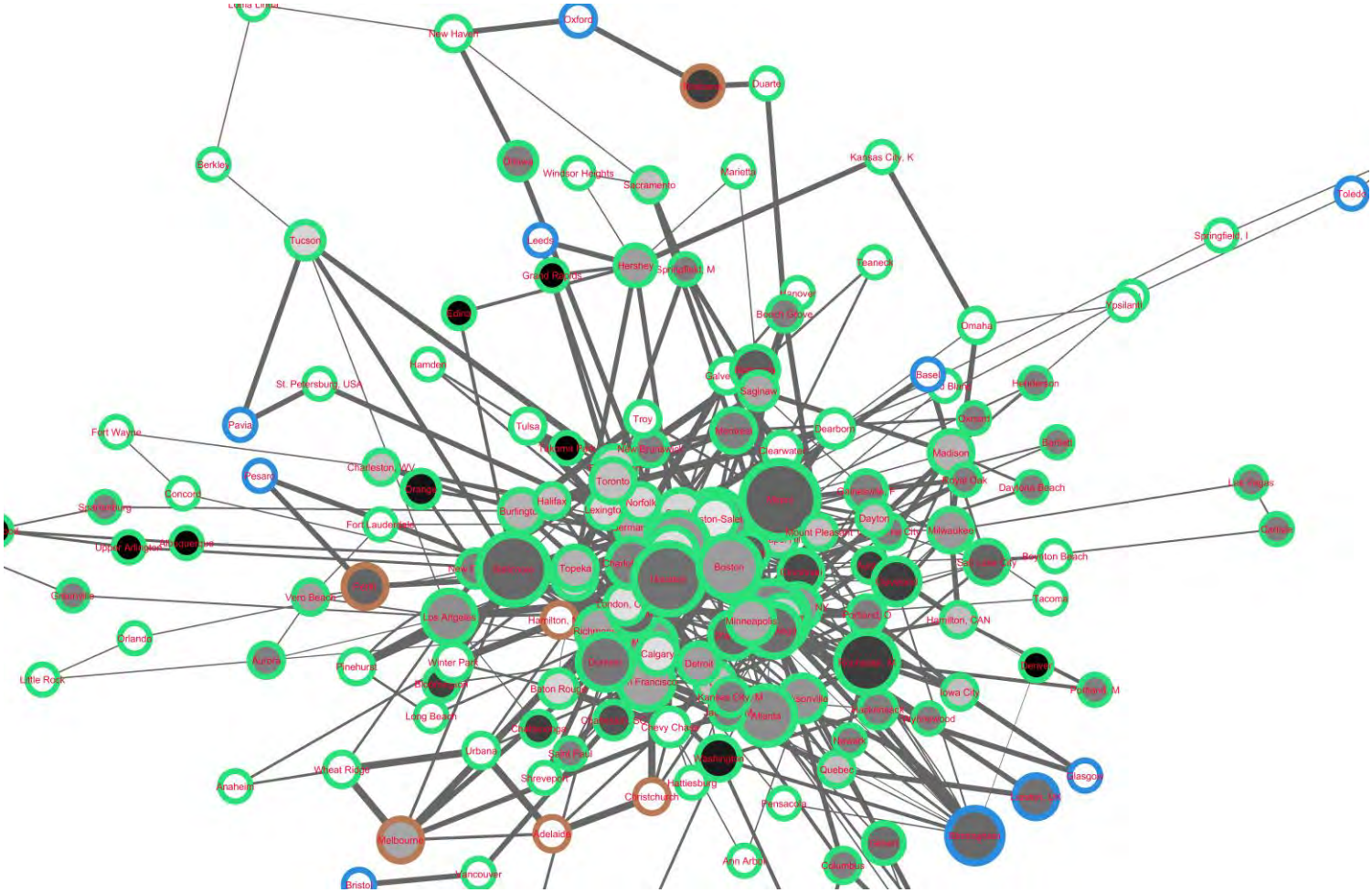


Figure I-7 : L'Océanie joue un rôle de connecteur entre l'Europe et l'Amérique du Nord. La couleur externe représente le continent (vert Amérique du Nord, bleu Europe, marron Océanie).

La moyenne du degré des villes d'Amérique du Nord est la plus élevée, 2 fois plus que l'Europe et 4 fois plus que l'Asie alors que le nombre d'études par ville est seulement différent entre Amérique du Nord et Europe (Table I-2). Ces statistiques nous permettent donc d'objectiver que le nombre d'études par centre en Asie n'est pas différent des autres continents, mais que ces villes ne travaillent pas en réseau (comme Seoul par exemple). Au contraire, les villes d'Amérique du Nord sont plus souvent en réseau, et travaillent également plus souvent en groupes (augmentation du coefficient de clustering). La maturité des études (score de phases) auxquelles participent les villes est plus avancée en Amérique du Nord et Europe par rapport à l'Amérique du Sud ou l'Asie (respectivement 0.62, 0.56 versus 0.16, 0.32). Amérique du Nord et Europe ont également les villes les plus avancées au niveau des essais cliniques puisqu'en moyenne 38.4 et 28.0% des études des villes sont terminées ; ce score est le plus faible en

Amérique du Sud et en Asie. Enfin, le support industriel des villes d'Amérique du Nord est significativement plus élevé que dans tous les autres continents atteignant 92.5% des villes.

Continent	Nombre de villes	Nombre d'études	Degree	Villes isolées	Score de phase	Etudes terminées (%)	Support industriel (%)
Afrique	3	3.0 ± 1.7	0 ± 0 **NA**	3 (100%) **O, E, NA**	0.27 ± 0.03	6.7 ± 11.5	0 ± 0 **NA**
Océanie	6	3.3 ± 1.5	3.7 ± 1.5 **SA**	0 (0%) **AF, SA**	0.72 ± 0.34 **A, SA**	15.0 ± 19.7	78.3 ± 34.9
Asie	66	4.0 ± 7.2	1.5 ± 1.8 **E, NA**	29 (44.6%) **E, NA**	0.32 ± 0.22 **E, O, NA**	15.5 ± 29.6 **NA**	50.8 ± 45.1 **NA**
Europe	112	2.3 ± 2.8 **NA**	2.6 ± 3.0 **A, NA**	22 (20.0%) **AF, A, NA, SA**	0.56 ± 0.35 **A, SA**	28.0 ± 40.0 **NA**	56.5 ± 45.3 **NA**
Amérique Nord	161	3.3 ± 3.1 **E**	5.8 ± 5.3 **AF, E, SA**	7 (4.4%) **A, AF, E, SA**	0.62 ± 0.31 **A, SA**	38.4 ± 35.2 **A, E, SA**	92.5 ± 22.3 **AF, A, E, SA**
Amérique Sud	9	2.1 ± 1.4	0.7 ± 1.0 **O, NA**	6 (66.7%) **E, O, NA**	0.16 ± 0.14 **E, O, NA**	3.7 ± 11.1 **NA**	16.7 ± 35.4 **NA**

Table I-2 : Description des propriétés mesurées dans le réseau, avec les études classifiées par continent. Les codes A, AF, AN, AS, E, O indiquent une différence significative des études pour le paramètre par rapport aux études des continents Asie, Afrique, Amérique du Nord, Amérique du Sud, Europe ou Océanie, respectivement.

La force de l'Amérique du Nord dans le réseau résiderait dans sa capacité à tisser des liens entre les différents centres, à agréger des financements industriels ; tout cela pourrait concourir à une capacité plus importante à terminer les études et à déposer de nouveaux dossiers réglementaires pour les phases suivantes. La faible avancée des pays asiatiques et d'Amérique du Sud nous interroge fortement.

Nous avons déterminé que les villes isolées avaient des études significativement de score de phase moins élevé et étaient moins terminées, moins supportées par l'industrie, plus retrouvées dans le continent Asiatique et dans les pays plus tolérants pour les ESCs. Ceci illustre certainement la difficulté de mettre en place des essais cliniques chez l'Homme et de se conformer aux procédures GMP nécessaires. L'industrie, via son financement, faciliterait le développement d'études multicentriques, et de fait la mise en commun des ressources, pour mener à bien les projets et augmenter plus vite en maturité.

Concernant la restriction des pays vis-à-vis des ESCs, il n'était pas judicieux d'observer le continent Amérique du Nord puisque 146 villes sur 159 concernent les USA (Table I-3). Degré,

maturité, et proportion d'études terminées, financement industriel et villes isolées sont donc augmentés avec l'augmentation de la restriction.

Restriction ESC Continent	+++	++	+	**Pas de politique**
Afrique	0	0	0	3
Océanie	0	2	4	0
Asie	0	5	39	21
Europe	33	56	20	1
Amérique Nord	146	10	2	1
Amérique Sud	0	6	0	2

Table I-3 : Illustration du poids que représente l'Amérique du Nord concernant la restriction vis-à-vis des cellules ESC. Pour tenter de s'affranchir de ce poids des USA, nous nous sommes centrés sur les pays Européens.

Pour tenter de s'affranchir de ce poids des USA, nous nous sommes centrés sur les pays Européens (Table I-3) ; les villes dont les pays sont très restrictifs ont un nombre d'études qui est diminué et un financement industriel qui est augmenté de manière significative lorsqu'on compare avec les villes dont les pays sont moins restrictifs. Un peu de souplesse quant au cadre législatif favoriserait ainsi l'émergence de nouvelles études ; lorsque cette souplesse diminue, le financement industriel s'avèrerait crucial pour pallier la lourdeur des procédures.

En regardant la distribution des différentes phases des études sur la Figure I-6, nous avons l'impression d'une distribution spatiale différentielle avec la présence des études plus blanches (Phase 3) et noires (Phase 0) à l'extérieur du réseau. Nous avons donc catégorisé ces villes selon leur maturité en Phase<0.33, Phase entre 0.33 et 0.66 et Phase>0.66 pour obtenir respectivement 118, 121 et 118 villes. Etant donné l'absence de distribution normale des données, nous avons utilisé le test de Dunn avec correction de Bonferroni afin d'estimer pour chaque catégorie la différence de moyenne entre les groupes avec leur significativité. Nous confirmons donc statistiquement notre impression visuelle (Figure I-8). En effet les centres dont les phases sont peu avancées se retrouvent plus éloignés, plus à l'écart des autres centres (diminution de betweenness et closeness centrality, augmentation de l'isolement), partageant moins de projets (diminution du degré, du coefficient de clustering, de la connectivité des voisins). Dans les phases plus élevées (troisième catégorie), le financement industriel est plus

important, soulignant les moyens plus importants nécessaires pour leur mise en place. Même si l'on note que ces centres participent moins en terme structurant au réseau (diminution du coefficient de clustering), la tendance à la connectivité des voisins plus élevée suggère que ces études sont plutôt multicentriques.

Il s'agit donc de la catégorie intermédiaire de phase, qui possède les caractéristiques de plus fort impact sur le réseau. Ces centres ont franchi les étapes en terme de maturité des études (des phases peu élevées à élevées). Il est également possible que durant les étapes de phase 1 le financement industriel soit encore dispensable.

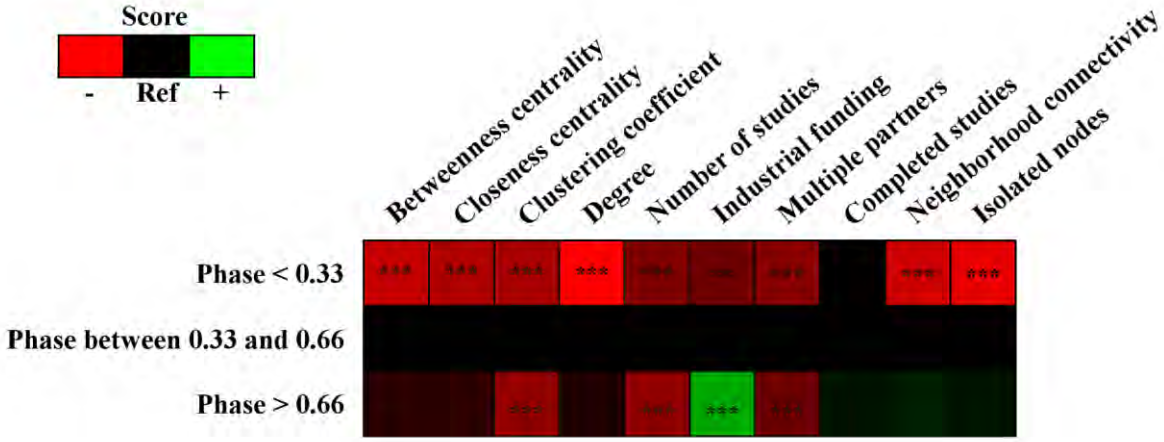


Figure I-8 : Catégorisation par le score de phase. En regardant la distribution des différentes phases des études sur la Figure I 6, nous avons l'impression d'une distribution spatiale différentielle avec la présence des études plus blanches (Phase 3) et noires (Phase 0) à l'extérieur du réseau. Nous avons donc catégorisé ces villes selon leur maturité en Phase<0.33, Phase entre 0.33 et 0.66 et Phase>0.66 pour analyser les caractéristiques des centres dans le réseau. La couleur verte ou rouge de la matrice est liée au test de Dunn, en prenant en référence le groupe phase entre 0.33 et 0.66. Le rouge implique un moins bon résultat/paramètre, et inversement pour le vert. La significativité est donnée par les étoiles (* $p<0.05$, ** $p<0.01$ et *** $p<0.001$).

Si l'on regarde l'évolution de la mise en place de ce réseau des CSMs, on s'aperçoit de l'existence de 3 temps distincts. Tout d'abord de 1999 à 2005, il s'agit du réseau AN qui s'implante. Jusqu'à 2008, l'AN croit et l'Europe émerge. Jusqu'en 2011, l'Europe et l'AN continuent de croitre alors que l'Asie émerge (Figure I-9).

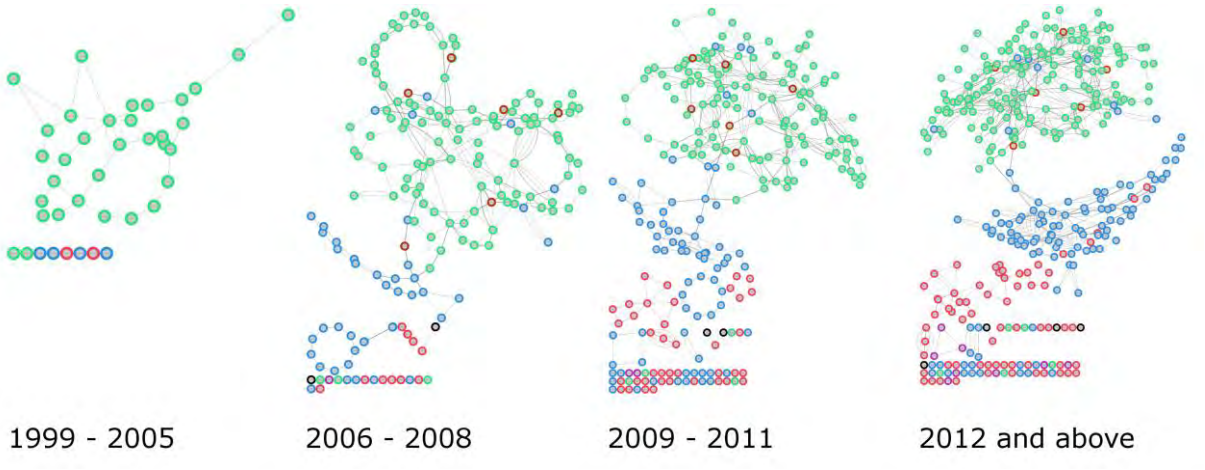


Figure I-9 : évolution temporelle de la mise en place du réseau. En vert l'Amérique du Nord, en bleue l'Europe, en rouge l'Asie, en violet l'Amérique du Sud, en noir l'Afrique et en marron l'Océanie.

Fraction hétérogène ou expandue, tissu adipeux ou moelle osseuse

Après avoir analysé les éléments de variation au sein d'un réseau, nous pouvons nous concentrer sur la comparaison entre les réseaux des caractéristiques. La Table I-4 compare dans la colonne de droite le réseau des CSMs (d'origine placentaire, menstruelle, ombilicale, moelle osseuse, dentaire, sans les ASCs) versus celui des ASCs. Nous pouvons observer que par rapport au réseau des ASCs, le réseau des autres CSMs a un coefficient de clustering, une hétérogénéité, une connectivité des voisins et une proportion de partenaires multiples plus élevés. Au contraire, la densité et la centralisation sont diminuées.

	Etudes avec CSMs (ASCs inclus)	**Etudes avec fraction hétérogène correspondante**	**Etudes avec CSMs (ASCs exclus)**	**Etudes avec ASCs**
Coefficient de clustering	0.091	0.062	0.186	0.050
Connectivité des voisins	3.552	1.692	3.000	2.000
Partenaires multiples	0.436	0.218	0.713	0.615
Noeuds isolés	56 (19.9%)	83 (38.8%)	68 (22.7%)	23 (24.2%)
Densité du réseau	0.010	0.008	0.010	0.021
Centralisation du réseau	0.061	0.030	0.064	0.098
Hétérogénéité du réseau	1.137	0.983	1.083	0.940

Table I-4 : Comparaisons des caractéristiques entre les réseaux.

Ceci illustre que le réseau des ASCs est encore en pleine structuration, avec un poids très important de peu de centres (Madrid et Séville sont les deux plus grands centres, au milieu du réseau, Figure I-10).

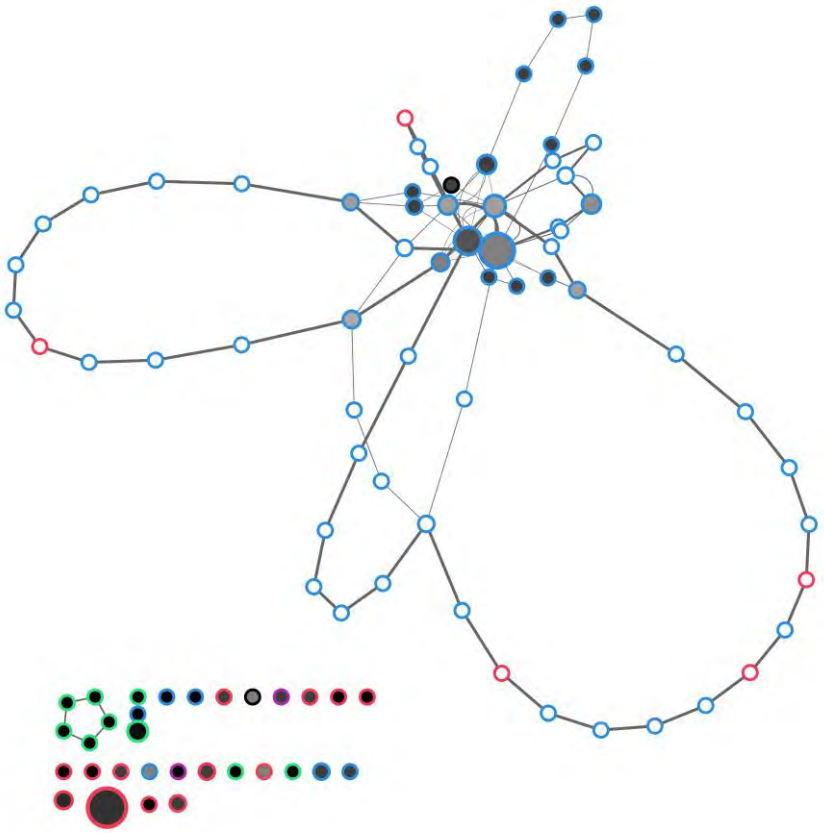


Figure I-10 : Réseau des ASCs avec un poids important de Madrid et Séville, au centre. Réseau avec un lien par étude dont la largeur est proportionnelle au nombre de patients dans l'essai clinique. Chaque ville (nœud) est représentée par un cercle dont le diamètre est proportionnel au nombre d'études auquel participe le centre. Le niveau de gris interne indique le score de phase (noir, phase 0 ; blanc, phase 3). La couleur externe représente le continent (rouge Asie, vert Amérique du Nord, bleu Europe, marron Océanie, jaune Afrique, violet Amérique du Sud). Il existe une force de type ressort entre les nœuds.

La colonne de gauche de la Table I-4 présente les différences entre le réseau de la fraction adhérente (CSMs, avec les ASCs inclus) versus la fraction hétérogène correspondante (fraction mononuclée, fraction stromale). Le réseau de la fraction hétérogène a tous les paramètres inférieurs à celui des CSMs. Le pourcentage de nœuds isolés est également plus élevé. Ceci illustre le fait que la fraction hétérogène fait l'objet de recherches plutôt mono-centriques. Cette absence de communication entre différents centres est peut-être le reflet de la plus grande facilité de mettre en œuvre ces procédures : la difficulté de mettre en place des procédures GMP

(« Good Manufacturing Practice ») pour les CSMs pourrait impliquer de se mettre à plusieurs pour répartir les tâches au sein de l'étude.

Une réflexion à avoir sur d'éventuels conflits d'intérêt et sur un marché parallèle

L'explosion de la thérapie cellulaire en dehors du cadre de la recherche cautionnée par les institutions académiques, inquiète de manière forte la communauté scientifique (67). Dans certains pays, l'absence de régulation stricte pourrait plutôt profiter à des cliniques privées, réalisant des greffes de cellules souches (fraction hétérogène ou des CSMs) sans avoir recours à de la recherche clinique. En effet, de nombreuses cliniques privées réalisent déjà des injections en routine. La stratégie de ces cliniques est de proposer des traitements autologues, de fraction hétérogène ou de cellules cultivées. L'aspect marketing est rodé : un aspect autologue qui rassure les patients, une désespérance souvent sur la pathologie ; le prix très important de la thérapeutique n'est donc plus au premier plan. Il existe une opacité importante quant à la manière dont sont préparées ces cellules ; elles ne sont sans doute pas si « non modifiées » que les industriels le prétendent (67). Lorsqu'on se rend sur le site de RNL Bio, (industriel implanté à Séoul dans la culture de cellules souches) un communiqué du 11 Avril 2013 nous informe que la société a développé une nouvelle technologie de culture permettant de rajeunir les cellules. Que mettent-ils dans leurs milieux de culture ? Comme l'utilisation clinique des cellules souches adultes est interdite en Corée, la société envoie ses patients au Japon et en Chine (67). La société Celltex a été fondée à partir de procédures développées par RNL Bio et son siège est situé au Texas. Le Texas n'autorise pas l'utilisation des CSMs en clinique mais autorise les patients à mettre les cellules en banque. Ceci a conduit Celltex à le notifier sur la page d'accueil de son site (Figure I-11).

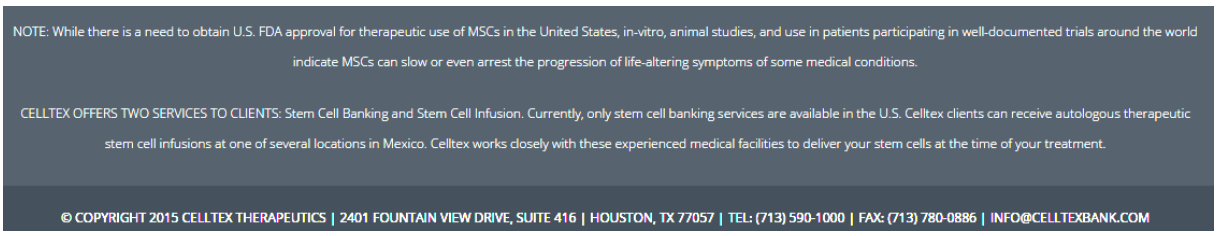


Figure I-11 : extrait d'un message affiché sur le site web de la société Celltex.

Néanmoins ces sociétés se sont lancées sur des essais cliniques qui ont été enregistrés dans ClinicalTrials.gov. Lorsque des méta-analyses seront réalisées pour analyser l'effet des CSMs en médecine régénérative, une attention toute particulière devra être portée sur la manière dont

les cellules seront obtenues, et sur la façon dont l'étude sera menée. En effet, certains praticiens travaillant pour ces sociétés arguent qu'il n'est pas nécessaire de mettre en place un placebo puisque le patient joue le rôle de son propre témoin en réalisant des études avant-après (67).

I.4.1 Conclusion

La puissance d'une telle approche nous permet d'envisager le suivi dynamique de la manière dont les communautés interagissent entre elles au niveau de la thérapie cellulaire.

Nous avons identifié une absence criante de coopération entre les différents pays et continents. L'hétérogénéité de la législation pourrait en être une des explications majeures. Pourtant, cette mise en commun des ressources permettrait d'aller plus vite et de gagner en maturité de phase des études.

Le rôle de l'industrie est également un aspect à prendre en considération. Le financement apporté en complément de l'académique est un élément important dans la structuration et la maturation des études. D'un autre côté, la possibilité d'une interférence de ces industriels dans les résultats des études nécessite une surveillance attentive des futures études publiées. Ceci est d'autant plus important que des sociétés, proposant de manière directe ou indirecte de la thérapie cellulaire de soin à leurs clients, se lancent maintenant dans des essais cliniques. Est-ce un *mea culpa* ou une façon de détourner l'attention sur ces pratiques ?

I.5 Protocole de MultiRevue de la littérature

A partir des projets précédents, nous avons éprouvé le besoin de formaliser ce concept de recherche intitulé MultiRevue (MultiReview). Comme nous l'avons évoqué, celui-ci se base sur une recherche systématique, exhaustive de la littérature, avec un protocole de recherche défini a priori, une procédure reproductible, le tout associé à un traitement facilité par l'outil informatique avec des outils de visualisation associés.

Principes généraux :

1. Établir un protocole a priori.

- Énoncer un objectif de recherche. Exemple : « L'objectif de cette MultiRevue est de lister et cartographier l'ensemble des maladies B_i ayant été rapportées comme associées au facteur de risque A ».
- Définir les informations à relever dans la grille d'extraction des données (exemple : référence pertinente (oui/non), type d'étude, significativité de l'association, niveau de preuve, année de publication, etc).
- Prévoir une session de concordance entre reviewers et un calcul de concordance inter-reviewers.

2. Définir un item principal A. Il peut s'agir d'un facteur de risque, mais aussi d'une maladie, d'une molécule ou de toute autre entité d'intérêt. Dans l'exemple cité en 1, l'item principal est le facteur de risque A.

3. Délimiter un périmètre de recherche [A] précis et reproductible pour cet item principal : choix des mots-clés, des options d'explosion, des opérateurs booléens, en rapport avec l'objectif de recherche.

4. Définir la nature des items multiples B_i. Il peut s'agir de maladies, mais aussi de facteurs de risque, de molécules ou de toutes autres entités d'intérêt. Dans l'exemple cité en 1, la nature des items multiples est la maladie, et la liste des maladies constitue B_i.

5. Délimiter un périmètre de recherche [B_i] précis et reproductible pour ces items multiples : choix des mots-clés, des options d'explosion, des opérateurs booléens, en rapport avec l'objectif de recherche. Le périmètre de recherche de l'item principal doit être exclusif du périmètre de recherche des items multiples B_i.

6. Croiser [A] et [B_i], en appliquant des filtres de recherche. Par exemple, ne sélectionner que les références pour lesquelles on dispose d'un résumé. Cette opération génère la base de données initiale, qui peut être exportée pour analyse.

7. Extraire les informations en fonction des champs créés dans la grille d'extraction des données.

8. Calculer le degré de concordance entre les reviewers. Résoudre les désaccords par consensus.

9. Générer les arbres et diagrammes de chorde en fonction des objectifs de recherche.

10. Discuter les résultats : perspectives de recherche et de santé publique.

I.6 Création d'un programme *ad hoc*

Le travail présenté dans ce chapitre fait l'objet d'un article en cours d'écriture.

Alors que nous avons développé un script Perl pour l'analyse des données CTD concernant les cellules souches, nous travaillons avec un groupe de programmeurs informatiques afin de développer un logiciel dédié à l'approche de la MultiRevue (MultiReview Manager ou MRM). Ci-dessous est détaillé le cahier des charges développé pour le projet, avec un système d'identification et de suivi de chaque item demandé.

[BDD1] Deux bases de données sont à interroger pour l'instant : PubMed et ClinicalTrials.gov. L'ajout de nouvelles bases de données se fait par la connaissance de la DTD spécifique.

[BDD2] Possibilité d'interroger les bases de données directement de l'application à l'aide des deux APIs (Application Programming Interface) respectives. Si cela n'est pas réalisable, la recherche sera effectuée directement dans la base de données, exportée en fichier XML puis réimportée dans MRM.

[BDD3] Le thésaurus utilisé est le thésaurus MeSH (actuellement en version 2016 et mis à jour hebdomadairement). Il est donc nécessaire de pouvoir le changer. Lors de l'importation des données de PubMed et ClinicalTrials.gov, il est nécessaire de faire le lien entre le thésaurus MeSH et les balises de MeSH fournies par ces deux bases de données. Donc chaque balise XML des bases de données est potentiellement un champ exploitable par MRM.

[BDD4] Travailler le MeSH avant la sélection des articles. Possibilité de ne même pas considérer certaines branches du MeSH avant de commencer. Exemple : on travaille avec la base de données PubMed mais on ne s'intéresse qu'à la branche [C - Diseases]. Ainsi le programme affichera les articles à choisir mais les mots clefs MeSH intégrés dans les articles n'appartiendront qu'à la branche [C].

[BDD5] Travailler le MeSH après la sélection des articles. Le MeSH est redondant. On pourra retrouver par exemple le VIH à la fois dans les maladies virales et dans les maladies du système immunitaire.

[BDD5a] Le programme devra identifier tous les doublons et demander lesquels garder.

[BDD5b] S'il reste des doublons, il faudra les identifier de manière claire (exemple 1/2 puis 2/2). Par exemple des problèmes de vessie sont retrouvés au niveau des systèmes uro-génitaux

des hommes et des femmes, il n'est pas possible de choisir entre les deux. Le diabète est également à la fois une maladie « Nutritionnelle et Métabolique » (C18) et du « système Endocrinien » (C19) ; il faudra donc garder ces deux doublons. Par contre, il peut être inutile de garder plusieurs fois le même mot clef dans une même branche ; il faudra donc choisir lequel ou lesquels éliminer.

[BDD6] Reclassement automatique du MeSH. Lorsqu'on importe des articles dans la base de données, on importe la manière dont les bases de données ont déjà attribué les bons mots clefs MeSH. Dans les deux bases de données, il y a les balises XML correspondantes. Néanmoins, lors du processus de sélection des articles, l'auteur peut avoir le besoin de changer la catégorie MeSH.

[GUI1] Présence d'une interface de connexion.

[GUI2] Permettre de créer des utilisateurs (nom, prénom, adresse mail, adresse, numéro de téléphone).

[GUI3] Rôle responsable de projet et rôle participant de projet. Les participants de projet peuvent voir l'équipe de travail. Les responsables de projet peuvent modifier l'équipe.

[GUI4] Outil de communication nécessaire entre les membres de l'équipe.

[PRJ1] Création du projet avec nom de projet, utilisateurs travaillant sur le projet, état d'avancement du projet.

[PRJ2] Paramétrer les questions à poser à l'utilisateur. Il faut pouvoir créer des questions libres (champs texte seul), des questions choix uniques ou des questions à choix multiples. Ces choix sont soit pour l'article (question posée une fois par article) soit pour le MeSH (question posée pour chaque article, autant de fois que de mots clefs MeSH sélectionnés). Attention, il faut raisonner ici non pas avec le code MeSH mais avec son libellé (exemple « Diabète » et non pas C18.452.394.750/C19.246)

[PRJ3] Pourcentage d'articles A% servant au coefficient de corrélation inter-auteurs. Ce pourcentage correspond au pourcentage d'articles que tous les auteurs auront en commun à analyser. A la fin de l'évaluation des articles, on regardera si les auteurs ont répondu de la même manière et on calculera le coefficient kappa.

[PRJ4] Pour chaque article à évaluer, plusieurs possibilités : soit accepter l'article, soit refuser l'article (possibilité de fournir la raison de ce refus), soit plus tard (l'article repassera plus tard),

soit demande d'aide (le cas de cet article sera tranché lors de la fusion des bases de données, lors du consensus des auteurs).

[PRJ5] Pour chaque base, il pourrait être intéressant de paramétrer quelles informations afficher lors de l'étape de sélection des articles. Par exemple, pour le projet X, je souhaite avoir le titre, le résumé et les mots clefs MeSH, pour le projet Y, le titre, le résumé et l'année de publication.

[PRJ6] Nécessité de pouvoir interrompre à n'importe quel moment le processus de sélection.

[PRJ7] Implémentation d'un diagramme de flux.

[PRJ8] Permettre de créer des scripts personnalisés afin de sélectionner une partie des données à reconstruire graphiquement. L'injection directe par l'utilisateur de code PHP n'est pas à exclure. Exemple : IF Champ1 == « ASC » THEN on garde les études ELSE on les exclue.

[PRJ9] Permettre d'ajouter de nouveaux articles à un projet.

[VIS1] Tous les mots clefs MeSH sélectionnés pour les articles devront se retrouver au bord du cercle de l'arbre.

[VIS2] Pour chaque mot clef MeSH, toute l'arborescence est ajoutée pour être enregistrée au centre du cercle. Par exemple si un article cite C01.256.278, il faudra ajouter au centre de l'arbre C01 et C01.256. La longueur de la branche sera juste liée aux nombres de divisions. Si C01.256.278 alors la longueur de branche sera égale à la division maximale retrouvée dans l'arbre divisée par 3. Attention aux mots clefs MeSH à la fois à l'intérieur et à l'extérieur du cercle.

[VIS3] Après chaque mot clef MeSH à l'extérieur du cercle, présence de graphiques empilés de différentes couleurs pour montrer la proportion des études en fonction de la question posée. Cela peut être seulement le nombre d'articles inclus, ou alors en fonction de catégories définies lors du processus de remplissage de la feuille d'extraction des données. Faire évoluer pour afficher autres choses que des graphiques empilés : des heatmaps, des graphiques linéaires…

[VIS4] Le fond de chaque mot clef MeSH à l'extérieur du cercle pourra être coloré en fonction de son niveau.

[VIS6] Reconstruction des connectogrammes. Principe de reconstruction graphique d'un tableau à 2 dimensions.

[EXP1] Export de statistiques par mot clef MeSH du bord du cercle, sous forme d'un site web. Exemple sur http://multireview.perso.sfr.fr.

[EXP2] Export également d'un fichier csv (ou xls/x) avec en ligne chaque article, en colonne les différentes variables relevées, y compris les articles refusés et pour quelle raison.

I.7 Conclusion

La force de notre approche est de pouvoir réaliser une analyse à la fois à forte (au niveau des champs d'application) et faible granulométrie (focus pathologique). Le message est renforcé par l'utilisation de représentations graphiques. Cette analyse pourrait être utile pour déterminer des lacunes dans la connaissance, des priorités de santé publique, déterminer les champs d'application où une augmentation des financements peut-être nécessaire, ou rationaliser les ressources (23).

Notre évaluation a également mis en évidence que le champ odonto-stomatologique était très peu représenté par l'utilisation des CSMs, même si cette problématique est actuellement en pleine expansion, en particulier dans le monde animal comme nous le détaillerons plus loin.

Par la mise à disposition de notre programme à la communauté scientifique, nous espérons que notre approche de MultiRevue fera des émules. Compte tenu de l'ampleur des grands volumes de données dans le monde scientifique et médical, j'ai suivi et obtenu plusieurs MOOCs (Annexe). Ceux-ci étaient notamment en rapport avec la gestion et l'interrogation des bases de données. Même si je n'ai pas mis directement en application ces enseignements dans le cadre de l'élaboration de cette MultiRevue, ceux-ci permettent de mieux comprendre les futurs enjeux numériques, et d'être plus à même de pouvoir suivre, guider l'équipe technique informatique, et interagir avec elle.

I.8 Bibliographie

1. Kiyindou A. De la fracture numérique à la fracture cognitive : pour une nouvelle approche de la société de l'information. In Thémat'IC 2007 « La maîtrise de l'information par les adultes : enjeux et méthodes », Strasbourg, 16 mars 2007 [en ligne]. Disponible sur : http://www.enssib.fr/bibliotheque-numerique/notice-1948.

2. Rubin R. Precision medicine: the future or simply politics? JAMA. 2015;313(11):1089-91.

3. Agence de la biomédecine – Etude comparative de l'encadrement juridique international. Octobre 2008.

4. Klitzman R, Appelbaum PS, Chung WK. Should life insurers have access to genetic test results? JAMA. 2014;312(18):1855-6.

5. Ashcroft R. Should genetic information be disclosed to insurers? No. BMJ. 2007;334(7605):1197.

6. Holm S. Should genetic information be disclosed to insurers? Yes. BMJ. 2007;334(7605):1196.

7. Jeannelle D. VNA : du rêve à la réalité. La Révolution Numérique Au Service De l'Hôpital de demain. Strasbourg, Juin 2013.

8. Kohane IS, Masys DR, Altman RB. The incidentalome: a threat to genomic medicine. JAMA. 2006;296(2):212-5.

9. Moses H, 3rd, Matheson DH, Cairns-Smith S, George BP, Palisch C, Dorsey ER. The anatomy of medical research: US and international comparisons. JAMA. 2015;313(2):174-89.

10. Zerhouni EA. Research funding. NIH in the post-doubling era: realities and strategies. Science. 2006;314(5802):1088-90.

11. Weber GM, Mandl KD, Kohane IS. Finding the missing link for big biomedical data. JAMA. 2014;311(24):2479-80.

12. Murad MH, Montori VM, Ioannidis JP, Jaeschke R, Devereaux PJ, Prasad K, et al. How to read a systematic review and meta-analysis and apply the results to patient care: users' guides to the medical literature. JAMA. 2014;312(2):171-9.

13. Lewington S, Clarke R, Qizilbash N, Peto R, Collins R. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. Lancet. 2002;360(9349):1903-13.

14. Bouvier M, Friedlander G. Appréhender la complexité du vivant à l'ère des « Omiques ». Med Sci (Paris). 2006;22(1):3-4.

15. De Bin R, Herold T, Boulesteix AL. Added predictive value of omics data: specific issues related to validation illustrated by two case studies. BMC Med Res Methodol. 2014;14:117.

16. Murdoch TB, Detsky AS. The inevitable application of big data to health care. JAMA. 2013;309(13):1351-2.

17. Charlet J, Cordonnier E, Gibaud B. Interopérabilité en médecine: quand le contenu interroge le contenant et l'organisation. Information-Interaction-Intelligence. 2002;2(2).

18. Bouamrane MM, Tao C, Sarkar IN. Managing interoperability and complexity in health systems. Methods Inf Med. 2015;54(1):1-4.

19. Landberg AH, Nguyen K, Pardede E, Rahayu JW. delta-dependency for privacy-preserving XML data publishing. J Biomed Inform. 2014;50:77-94.

20. Huang EW, Wang DW, Liou DM. Development of a deterministic XML schema by resolving structure ambiguity of HL7 messages. Comput Methods Programs Biomed. 2005;80(1):1-15.

21. Database resources of the National Center for Biotechnology Information. Nucleic Acids Res. 2015;43(Database issue):D6-17.

22. Guo YW, Tang YT, Kao HY. Genealogical-based method for multiple ontology self-extension in MeSH. IEEE Trans Nanobioscience. 2014;13(2):124-30.

23. Tasneem A, Aberle L, Ananth H, Chakraborty S, Chiswell K, McCourt BJ, et al. The database for aggregate analysis of ClinicalTrials.gov (AACT) and subsequent regrouping by clinical specialty. PLoS One. 2012;7(3):e33677.

24. Riveros C, Dechartres A, Perrodeau E, Haneef R, Boutron I, Ravaud P. Timing and Completeness of Trial Results Posted at ClinicalTrials.gov and Published in Journals. PLoS Med. 2013;10(12):e1001566.

25. Hayrinen K, Saranto K, Nykanen P. Definition, structure, content, use and impacts of electronic health records: a review of the research literature. Int J Med Inform. 2008;77(5):291-304.

26. Fayyad U, Piatetsky-Shapiro G, P S. From Data Mining to Knowledge Discovery in Databases. AI Magazine. 1996;17(3):37-54.

27. Wong B. Points of view: Visualizing biological data. Nat Meth. 2012;9(12):1131-.

28. Gough D, Thomas J, Oliver S. Clarifying differences between review designs and methods. Syst Rev. 2012;1(1):28.

29. Trounson A, Thakar RG, Lomax G, Gibbons D. Clinical trials for stem cell therapies. BMC Med. 2011;9:52.

30. Rossant J. Stem cells from the Mammalian blastocyst. Stem Cells. 2001;19(6):477-82.

31. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, et al. Embryonic stem cell lines derived from human blastocysts. Science. 1998;282(5391):1145-7.

32. Hauser SL. Hematopoietic stem cell transplantation for MS: extraordinary evidence still needed. Jama. 2015;313(3):251-2.

33. Harousseau JL, Moreau P. Autologous hematopoietic stem-cell transplantation for multiple myeloma. N Engl J Med. 2009;360(25):2645-54.

34. Medvinsky A, Livesey FJ. On human development: lessons from stem cell systems. Development. 2015;142(1):17-20.

35. Collins FS. Exceptional opportunities in medical science: a view from the National Institutes of Health. Jama. 2015;313(2):131-2.

36. Murphy MB, Moncivais K, Caplan AI. Mesenchymal stem cells: environmentally responsive therapeutics for regenerative medicine. Exp Mol Med. 2013;45:e54.

37. Ilic D, Polak J. Stem cell based therapy--where are we going? Lancet. 2012;379(9819):877-8.

39. Squillaro T, Peluso G, Galderisi U. Clinical Trials with Mesenchymal Stem Cells: An Update. Cell Transplant. 2015.

40. Atala A. Human embryonic stem cells: early hints on safety and efficacy. The Lancet.379(9817):689-90.

41. Aldhous P. A world of difference. Nature. 2001;414(6866):838.

42. Nisbet MC. The Competition for Worldviews: Values, Information, and Public Support for Stem Cell Research. International Journal of Public Opinion Research. 2005;17(1):90-112.

43. Ren G, Chen X, Dong F, Li W, Ren X, Zhang Y, et al. Concise review: mesenchymal stem cells and translational medicine: emerging issues. Stem Cells Transl Med. 2012;1(1):51-8.

44. Ilic D. Trends in the stem cell and regenerative medicine industry. Regen Med. 2012;7(5):645-8.

45. van der Spoel TI, Jansen of Lorkeers SJ, Agostoni P, van Belle E, Gyongyosi M, Sluijter JP, et al. Human relevance of pre-clinical studies in stem cell therapy: systematic review and meta-analysis of large animal models of ischaemic heart disease. Cardiovasc Res. 2011;91(4):649-58.

46. Papazova DA, Oosterhuis NR, Gremmels H, van Koppen A, Joles JA, Verhaar MC. Cell-based therapies for experimental chronic kidney disease: a systematic review and meta-analysis. Dis Model Mech. 2015;8(3):281-93.

47. Francis DP, Mielewczik M, Zargaran D, Cole GD. Autologous bone marrow-derived stem cell therapy in heart disease: discrepancies and contradictions. Int J Cardiol. 2013;168(4):3381-403.

48. Nowbar AN, Mielewczik M, Karavassilis M, Dehbi HM, Shun-Shin MJ, Jones S, et al. Discrepancies in autologous bone marrow stem cell trials and enhancement of ejection fraction (DAMASCENE): weighted regression and meta-analysis. Bmj. 2014;348:g2688.

49. Fisher SA, Brunskill SJ, Doree C, Mathur A, Taggart DP, Martin-Rendon E. Stem cell therapy for chronic ischaemic heart disease and congestive heart failure. Cochrane Database Syst Rev. 2014;4:Cd007888.

50. Clifford DM, Fisher SA, Brunskill SJ, Doree C, Mathur A, Watt S, et al. Stem cell treatment for acute myocardial infarction. Cochrane Database Syst Rev. 2012;2:Cd006536.

51. Jeong H, Yim HW, Cho Y, Park HJ, Jeong S, Kim HB, et al. The effect of rigorous study design in the research of autologous bone marrow-derived mononuclear cell transfer in patients with acute myocardial infarction. Stem Cell Res Ther. 2013;4(4):82.

52. Pavo N, Charwat S, Nyolczas N, Jakab A, Murlasits Z, Bergler-Klein J, et al. Cell therapy for human ischemic heart diseases: Critical review and summary of the clinical experiences. J Mol Cell Cardiol. 2014;75c:12-24.

53. Lu D, Chen B, Liang Z, Deng W, Jiang Y, Li S, et al. Comparison of bone marrow mesenchymal stem cells with bone marrow-derived mononuclear cells for treatment of diabetic critical limb ischemia and foot ulcer: a double-blind, randomized, controlled trial. Diabetes Res Clin Pract. 2011;92(1):26-36.

54. da Silva Meirelles L, Chagastelles PC, Nardi NB. Mesenchymal stem cells reside in virtually all post-natal organs and tissues. J Cell Sci. 2006;119(Pt 11):2204-13.

55. Friedenstein AJ, Chailakhjan RK, Lalykina KS. The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. Cell Tissue Kinet. 1970;3(4):393-403.

56. Bourin P, Sensebé L, Planat-Bénard V, Roncalli J, Bura-Rivière A, Casteilla L. Culture and Use of Mesenchymal Stromal Cells in Phase I and II Clinical Trials. Stem Cells Int. 2010;2010:503593.

57. Philippe B, Luc S, Valerie PB, Jerome R, Alessandra BR, Louis C. Culture and Use of Mesenchymal Stromal Cells in Phase I and II Clinical Trials. Stem Cells Int. 2010;2010:503593.

58. Casteilla L, Planat-Benard V, Laharrague P, Cousin B. Adipose-derived stromal cells: Their identity and uses in clinical trials, an update. World J Stem Cells. 2011;3(4):25-33.

59. Dominguez-Bendala J, Lanzoni G, Inverardi L, Ricordi C. Concise review: mesenchymal stem cells for diabetes. Stem Cells Transl Med. 2012;1(1):59-63.

60. Kern S, Eichler H, Stoeve J, Kluter H, Bieback K. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. Stem Cells. 2006;24(5):1294-301.

61. Griffin MD, Elliman SJ, Cahill E, English K, Ceredig R, Ritter T. Concise review: adult mesenchymal stromal cell therapy for inflammatory diseases: how well are we joining the dots? Stem Cells. 2013;31(10):2033-41.

62. Wei X, Yang X, Han ZP, Qu FF, Shao L, Shi YF. Mesenchymal stem cells: a new trend for cell therapy. Acta Pharmacol Sin. 2013;34(6):747-54.

63. Scott J, Tallia A, Crosson JC, Orzano AJ, Stroebel C, DiCicco-Bloom B, et al. Social network analysis as an analytic tool for interaction patterns in primary care practices. Ann Fam Med. 2005;3(5):443-8.

64. Goh KI, Cusick ME, Valle D, Childs B, Vidal M, Barabasi AL. The human disease network. Proc Natl Acad Sci U S A. 2007;104(21):8685-90.

65. Dhar D, Hsi-en Ho J. Stem Cell Research Policies around the World. The Yale Journal of Biology and Medicine. 2009;82(3):113-5.

66. Califf RM, Zarin DA, Kramer JM, Sherman RE, Aberle LH, Tasneem A. Characteristics of clinical trials registered in ClinicalTrials.gov, 2007-2010. Jama. 2012;307(17):1838-47.

67. Cyranoski D. Stem-cell therapy takes off in Texas. Nature. 2012;483(7387):13-4.

Partie II

Parodontite et régénération par l'utilisation des CSMs

Table des illustrations

II. Parodontite et régénération par l'utilisation des CSMs

II.1 La parodontite

II.1.1 Définition et épidémiologie

La parodontite est une maladie immuno-infectieuse caractérisée par la destruction du système d'ancrage de la dent ; le parodonte profond (cément, os alvéolaire et ligament desmodontal), qui conduit à la formation de l'entité pathognomonique de la maladie, la poche parodontale (Figure II-1). Sans prise en charge thérapeutique, le processus aboutit à une mobilité de la dent, et au final à sa perte (1).

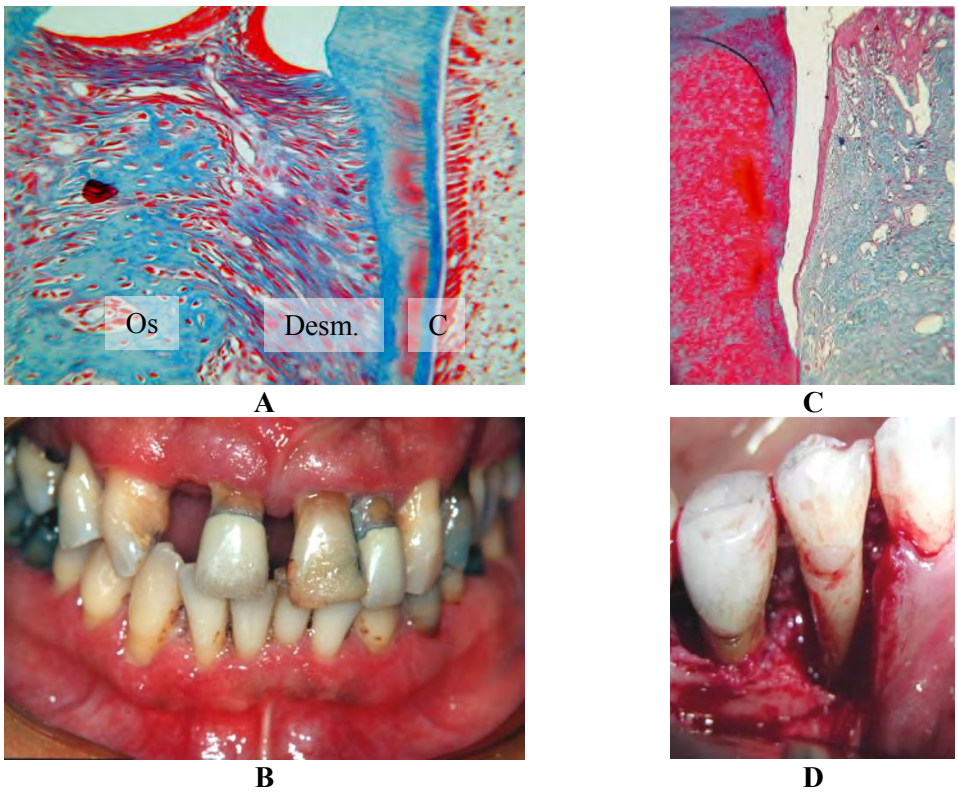


Figure II-1 : lésions parodontales, clinique et histologie. **A-** coupe histologique d'un parodonte sain, avec le parodonte profond : l'os alvéolaire (Os), le ligament desmodontal (desm.) et le cément (C). B- Photographie d'un patient présentant une parodontite. **C –** Coupe histologique d'une lésion parodontale (Pr Brunel). **D –** Photographie d'une lésion parodontale infra-osseuse après décollement d'un lambeau d'accès (Dr Barthet, Toulouse).

Les définitions cliniques de la parodontite diffèrent selon les études (2) et la littérature révèle une absence de consensus quant à une définition dans les études épidémiologiques (3). Bien que la prévalence estimée varie selon la manière dont la maladie est définie et la tranche d'âge

considérée, la parodontite affecte en moyenne 35 à 50% des adultes dans les pays développés (4, 5).

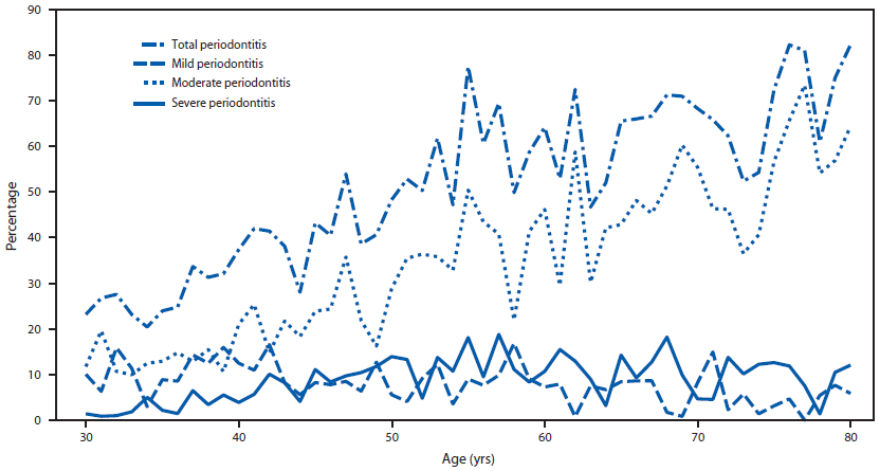


Figure II-2 : évolution de la prévalence suivant les tranches d'âge (inspiré d'Eke et al. (6) et illustration de A. Martegoutte)

D'un point de vue étiopathogénique et physiopathologique, la réponse inflammatoire chronique et l'infection locale, induisent la mise en place d'un cercle vicieux de destruction tissulaire. Bien que les bactéries parodonto-pathogènes représentent à elles seules un déterminant étiologique fondamental de la parodontite, un élément essentiel de la progression de la maladie est la capacité de l'hôte à mettre en place une réponse immunitaire efficace. Elle est conditionnée notamment par la sécrétion de cytokines, l'activation des clastes, le recrutement des cellules inflammatoires, mais aussi les communications avec le stroma (7).

II.1.2 Biofilms bactériens

La bouche présente approximativement 700 espèces bactériennes ; entre 100 et 200 espèces différentes sont présentes dans une bouche saine, organisées en différents écosystèmes buccaux, pathogènes ou commensaux (8, 9).

La survie des bactéries orales requiert leur adhésion aux surfaces dentaires et épithéliales. En effet, les flux sécrétoires (fluide gingival et flux salivaire) empêchent la croissance des bactéries planctoniques tant qu'elles ne sont pas agrégées en biofilm (8). Celui-ci est constitué de communautés de bactéries qui résident à l'intérieur d'une matrice de polymères extracellulaires

(PEC)(10). Les micro-organismes comptent pour seulement 10% de la masse sèche du biofilm. Les 90% restants sont constitués par la matrice, formée d'exopolysaccharides, de lipides (dont les lipopolysaccharides)(11). La nature des PEC conditionne l'architecture tridimensionnelle des biofilms ainsi que leur adhésion aux structures et leur cohésion. De plus, leur composition est influencée par l'environnement (pH, concentration calcique) et par les micro-organismes présents (11).

De fait, les bactéries sont protégées à l'intérieur du biofilm ; d'une part par une barrière physique contre les agents biocides et les activités enzymatiques, et d'autre part, par l'existence de zones dans lesquelles les bactéries sont quiescentes, en phase stationnaire, dans un état de résistance augmentée (10). En biofilm, la résistance bactérienne aux agents antimicrobiens peut-être jusqu'à 1000 fois plus importante que sous la forme planctonique (12). Ainsi, il est nécessaire de développer de nouvelles thérapies plus actives sur les biofilms, qui pourraient être capables à la fois de les désorganiser et d'avoir une activité biocide.

La plupart des pathologies de la sphère orale est d'origine infectieuse et liée à des bactéries pathogènes organisées en biofilms comme les lésions carieuses, parodontites, pathologies pulpaires, candidose (13).

II.1.3 Parodontite et écologie bactérienne

La physiopathologie des maladies infectieuses d'origine bactérienne implique souvent le respect des postulats de Koch (1890) qui prônent la spécificité bactérienne. Une bactérie engendre une maladie, et le fait d'administrer cet agent infectieux à un individu sain induit la pathologie. C'est notamment le cas de la tuberculose dont l'agent pathogène est *Mycobacterium tuberculosis* ou de la maladie de Lyme avec *Borrelia burgdorferi*. La parodontite n'obéit pas à ces postulats comme Miller (élève de Koch) en avait eu l'intuition, en essayant d'isoler la bactérie responsable de la « pyorrhée alvéolo-dentaire», sans succès (14).

La formation du biofilm nécessite une première étape d'adhésion de colonisateurs initiaux grams positifs (*Streptococci* et *Actinomyces*) aux protéines et glycoprotéines de la salive, adsorbés aux surfaces dentaires. La seconde étape est l'adhérence aux bactéries : coagrégation et coadhésion. Les bactéries du genre *Fusobacterium* sont des bactéries clefs car elles sont capables, plus que les autres genres, de coagréger avec des colonisateurs initiaux, secondaires et tardifs. Chez les individus sains, *Streptococci*, *Actinomyces* et *Veillonellae* prédominent alors

que les parodontopathogènes sont présents en faible quantité. Avec l'évolution de la pathologie, on observe un changement spatial et temporel de l'écologie bactérienne vers des complexes bactériens grams négatifs pathogènes (complexes dits orange et rouge), et ce d'autant plus que les poches augmentent en profondeur (15)(Figure II-3). Les méthodes moléculaires d'identification (pyroséquençage de l'ARN 16S par exemple) permettent d'envisager une analyse plus approfondie du microbiome, la détermination de groupes bactériens sains, à risques ou pathologiques, que les souches soient cultivables ou non (16, 17).

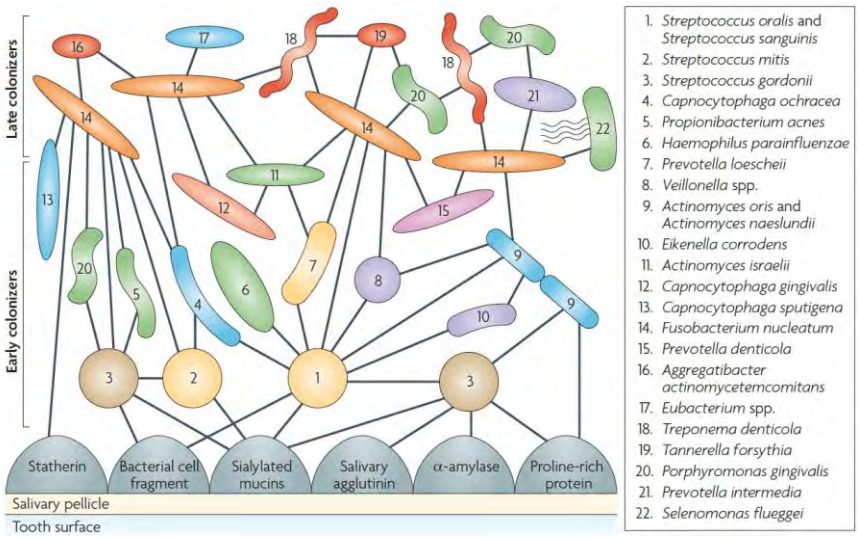


Figure II-3 : Schéma de l'écologie bactérienne de la plaque bactérienne

Une hygiène orale insuffisante est un facteur de risque de développer une parodontite ; mais une hygiène dentaire défaillante, même sur le long terme, n'induira pas forcement des lésions parodontales chez l'hôte. Abdelatif et al., en analysant des données de l'étude National Health and Nutritional Examination Survey I (18), fait la constatation que s'il existe une association significative entre parodontite et hygiène bucco-dentaire, la proportion d'individus ayant une hygiène déficiente et sans parodontite est d'environ 50% (détails par tranche d'âge après 40 ans dans les carrés latins ci-dessous). De manière encore plus étonnante, environ 4% des individus avec une excellente hygiène, développe une parodontite. Une conclusion similaire a été formulée par Löe *et al.* dans une étude longitudinale (19) : il a ainsi pu mettre en évidence un groupe de sujets avec une hygiène buccale déficiente et une gingivite, mais avec absence de parodontite.

Après 40 ans	*Hygiene* +	*Hygiene* -
Periodontitis +	N=200 (3.9%)	N=1111 (46.7%)
Periodontitis -	N=4936 (96.1%)	N=1268 (53.3%)

Après 50 ans	*Hygiene* +	*Hygiene* -
Periodontitis +	N=172 (4.2%)	N= 743 (49%)
Periodontitis -	N=3872 (95.8%)	N=773 (51%)

Après 60 ans	*Hygiene* +	*Hygiene* -
Periodontitis +	N=133 (4.3%)	N=469 (50.3%)
Periodontitis -	N=2996 (95.7%)	N=463 (49.7%)

Tableau II-1 : tableau récapitulatif du carré latin hygiène et parodontite, d'après Abdellatif et al. (18)

Mais les bactéries, même parodonto-pathogènes, sont également détectées à l'état physiologique ; elles peuvent même traverser la barrière épithéliale et coloniser le conjonctif gingival, mais sont alors éliminées par l'immunité sentinelle tant qu'elle est compétente (20). Le microbiote doit être considéré comme un organe métabolique, capable de moduler les fonctions de l'hôte (21). Bien que peu d'éléments expérimentaux aient pu mettre en évidence une défaillance dans ce système, la clinique nous montre qu'il peut exister chez certains sujets une rupture de cet équilibre. Dès lors, il est possible de penser que nous sommes inégaux face au développement de la parodontite et de chercher d'autres étiologies possibles. Nous pouvons suggérer que chaque individu aurait sa propre tolérance face aux agents pathogènes : chez certains, même une faible dose de ces agents infectieux suffirait à déclencher une rupture de l'équilibre précaire au sein du tissu parodontal, et induire un cycle de destruction tissulaire.

II.1.4 Parodontites et immunité

Il est communément admis que la parodontite est une polyinfection dans laquelle charge bactérienne et immunité sont alternativement à l'origine de la destruction et de tentatives de réparations tissulaires, typiques de l'inflammation chronique (22, 23).

Comme évoqué dans le paragraphe précédent, l'impact du déséquilibre de l'écosystème bactérien pourrait constituer l'élément déclenchant. Au contraire, l'hôte pourrait avoir un

terrain propice à la rupture de l'homéostasie, très certainement à cause d'une « dysimmunité locale » (bien qu'aucun auteur n'ait jamais pu la mettre formellement en évidence encore).

Certains auteurs pensent qu'il n'est même pas possible de générer une réponse de protection contre les parodonto-pathogènes sans induire de réponse destructrice contre le parodonte (23). Le déséquilibre de la balance entre métallo-protéinases (MMP) et inhibiteurs de métallo protéinases (TIMP) mais également de la balance entre le *receptor activator of nuclear factor κB* (RANK), son ligand (RANKL) et sa contrepartie soluble (OPG), induit une destruction tissulaire. RANKL se liant à RANK présent à la surface des pré-ostéoclastes stimule leur activation, maturation et activité ostéolytique (23). La dysbiose parodontale entraine un recrutement massif initial de polynucléaires neutrophiles (24). Le milieu est alors le siège d'une production massive de radicaux libres par les neutrophiles dont le nombre est positivement corrélé à la sévérité de la parodontite (25). L'expression des cytokines pro-inflammatoires par les macrophages a également pour effet d'inhiber la différenciation ostéogénique (26).

La reconnaissance des parodonto-pathogènes par les *toll-like receptors* (TLR, en particulier TLR-2 et TLR-4), les systèmes *nucleotide-binding oligomerization domain receptors* (NOD) et de l'inflammasome, participent à l'activation du système de défense inné et à la production de cytokines inflammatoires comme le TNF-α, l'IL-1 et l'IL-6 (23). La production et l'activation de certaines protéines (système du complément, défensines, lactoferrine), participent de manière directe et indirecte à la défense antibactérienne (23). Le système immunitaire adaptatif est également mis en jeu avec un rôle des sous-groupes lymphocytaires Th1 et Th17 dans la destruction osseuse, en particulier par les cytokines pro-inflammatoires (IL-1β, TNF-α, IL-6, IL-17 et IFN-γ). Au contraire, les sous-groupes lymphocytaires Th2 et Tregs ont un rôle de contrôle et d'atténuation de la progression de la parodontite (bien que ce rôle soit quelque peu controversé pour les Th2) (23). Cet effet est en partie médié par les cytokines anti-inflammatoires IL-4, IL-10 et TGF-β (23).

Les parodonto-pathogènes développent également des stratégies afin d'échapper à la réponse immunitaire parodontale. En effet *Porphyromonas gingivalis* est capable de co-activer chez les neutrophiles, via ses *pathogen associated molecular patterns* (PAMPs) et ses protéases, les voies du TLR2 et du récepteur au C5a (C5aR). L'interférence de ces deux voies de signalisation va entrainer, d'une part l'ubiquitination et la dégradation protéosomale de MyD88 inhibant la réponse antimicrobienne, et d'autre part RhoA supprimant ainsi la phagocytose (27). Cette inhibition de la réponse antimicrobienne est également retrouvée avec *Prevotella intermedia*, s'opposant au complément grâce aux protéases qu'elle exprime (28) ou *Porphyromonas*

gingivalis capable de supprimer l'endocytose de *Fusobacterium nucleatum* par les macrophages (29).

La physiopathologie de la parodontite implique donc une régulation très fine au sein des tissus. Son traitement doit mettre en jeu une action antibactérienne efficace contre les biofilms ainsi qu'un contrôle de l'inflammation.

Les parodontites sont donc potentiellement à la fois un foyer infectieux et inflammatoire, pouvant être le reflet d'une susceptibilité individuelle. Nous allons donc nous intéresser dans la suite de ce travail au lien qu'il peut exister entre parodontite et pathologies systémiques.

II.1.5 Une cartographie des essais cliniques en médecine parodontale

II.1.5.1 Article 3: "Clinical research activity in periodontal medicine: a systematic mapping of trial registers"

Le travail présenté dans ce chapitre est accepté pour publication sous réserve de modifications mineures dans « Journal of Clinical Periodontology ».

Contexte

L'idée que la parodontite pourrait être associée à la santé générale remonte à l'antiquité, où déjà Hippocrate avait reporté deux cas de patients chez lesquels l'éradication des foyers infectieux buccaux avait résolu des symptômes articulaires de type rhumatismaux (30). Si ce sujet était populaire auprès de la communauté scientifique au 20ème siècle (théorie de l'infection focale)(31), il est par la suite resté en dormance (32). Ce n'est que plus récemment, dans les années 1990, que le sujet du lien entre parodontite et maladies systémiques retrouve un regain d'intérêt au travers d'une nouvelle branche de la parodontologie « la médecine parodontale » (33). Compte tenu d'une part de la morbidité et des coûts engendrés par les pathologies générales, d'autre part de la prévalence élevée de la parodontite en population générale, de nombreux espoirs ont été placés dans le traitement parodontal.

La parodontite peut être un marqueur de risque général comme cela est envisagé à l'heure actuelle pour la naissance d'enfants prématurés et de faible poids de naissance. Les femmes enceintes présentant une parodontite sont plus à risque de donner naissance à un enfant prématuré que des femmes enceintes sans (34) ; mais le traitement parodontal n'améliore pas

ces paramètres (35). Au contraire, la relation est considérée causale pour le diabète, et le traitement parodontal permettrait d'obtenir une baisse moyenne de 0.7% du paramètre de surveillance de cette pathologie (36), l'Hba1c (hémoglobine glyquée). Néanmoins, les relations causales ne sont toujours pas clairement établies (1), et des études récentes publiées dans de prestigieux journaux ne mettent pas en évidence d'effet significatif du traitement parodontal (37, 38).

Les registres des essais cliniques s'étant mis en place à partir des années 1990, leur exploration permet d'avoir une vision globale de la médecine parodontale. Le but de ce travail est de décrire les conditions systémiques qui ont été associées à la maladie parodontale.

Méthodologie

La base mondiale des essais cliniques a été utilisée (regroupant 16 sources de registres, incluant CTD) : « l'International Clinical Trials Registry Platform » (ICTRP). Cette base de données, du fait de la diversité de ses sources, est beaucoup moins bien tenue, elle possède moins d'informations que CTD, ne possède pas d'automatisation de classement MeSH et est moins bien structurée (39). Nous avons ainsi choisi d'extraire les informations manuellement et de classifier nous-même les pathologies dans le MeSH.

La stratégie de recherche était "periodont* OR gingiv* OR *implantitis", dernièrement conduite le 27/05/2015.

Résultats principaux

Sur les 966 résultats, 142 études ne correspondaient pas aux critères d'inclusion et ont été exclues. Au final, nous avons identifié 242 études à propos de la médecine parodontale.

57 conditions systémiques ont été associées aux maladies parodontales soit environ 2% des branches « Diseases [C] » et « Phenomena and Processes [G] » du MeSH ; nous avons observé au fur et à mesure du temps une diversification des conditions systémiques testées. Si les relations entre parodontite et diabète, maladies cardio-vasculaires ou complications durant la grossesse sont des sujets actifs et prolifiques, d'autres hypothèses d'association sont en cours de développement comme pour l'anémie, les pathologies du foie, dyspepsies ou la spondylarthrite ankylosante.

Article

Journal of Clinical Periodontology
Official Journal of The European Federation of Periodontology
Founded by the British, Dutch, French, German, Scandinavian, and Swiss Societies of Periodontology

Clinical research activity in periodontal medicine: a systematic mapping of trial registers

Journal:	*Journal of Clinical Periodontology*
Manuscript ID	CPE-11-15-6021.R1
Manuscript Type:	Systematic Review
Date Submitted by the Author:	05-Jan-2016
Complete List of Authors:	Monsarrat, Paul; Faculté de chirurgie dentaire, ; CNRS 5273; UMR STROMALab; Université de Toulouse UPS; INSERM U1031; EFS Pyrénées – Méditerranée; BP 84225, F-31 432, Blaizot, Alessandra; Faculté de chirurgie dentaire de Lille 2, Kémoun, Philippe; Faculté de chirurgie dentaire, Ravaud, Philippe; Université Paris Descartes, Faculté de Médecine & INSERM UMR-1153, ; Department of Epidemiology, Columbia University Mailman School of Public Health, Nabet, Cathy; Faculté de chirurgie dentaire, ; INSERM U1027, Sixou, Michel; Faculté de chirurgie dentaire, Vergnes, Jean-Noël; Faculté de chirurgie dentaire, ; Division of Oral Health and Society, Faculty of dentistry, McGill University,
Topic:	Aetiology
Keywords:	Periodontal Diseases, Periodontal Medicine, Systematic Review, Clinical Trials, periodontitis
Main Methodology:	Systematic Review

Clinical research activity in periodontal medicine: a systematic mapping of trial registers

Running title

Periodontal medicine in trial registers

Authors

Paul Monsarrat [1], Alessandra Blaizot [2], Philippe Kémoun [3], Philippe Ravaud [4], Cathy Nabet [5], Michel Sixou [6], Jean-Noel Vergnes [7]

1: Paul Monsarrat, DDS (corresponding author)
Paul Sabatier University, Dental Faculty, Department of Anatomical Sciences and Radiology, Toulouse University Hospital, Toulouse, France.
&
CNRS 5273; UMR STROMALab; University of Toulouse UPS; INSERM U1031; EFS Pyrenees – Mediterranean; Toulouse, France.

Mail address : UFR Odontologie de Toulouse
3, chemin des maraîchers
31062 Toulouse Cedex 9.
E-mail address: paul.monsarrat@gmail.com
Telephone number: +33 5 62 17 29 29
Fax number: +33 5 61 25 47 19

2: Alessandra Blaizot, DDS
Lille 2 University, Dental Faculty, Department of Public Health, Lille, France.
E-mail address: alessandra.blaizot@wanadoo.fr

3: Philippe Kémoun, PhD, DDS
Paul Sabatier University, Dental Faculty, Department of Biological Sciences, Toulouse University Hospital, Toulouse, France.
E-mail address: philippe.kemoun@univ-tlse3.fr

4: Philippe Ravaud, PhD, DDS
Paris Descartes University, Faculty of Medicine, Paris, France
&
INSERM UMR-1153, Paris, France
&
Department of Epidemiology, Columbia University Mailman School of Public Health, New York, NY, USA
E-mail address: philipperavaud@gmail.com

5: Cathy Nabet, PhD, DDS

Paul Sabatier University, Dental Faculty, Department of Epidemiology and Public Health, Toulouse University Hospital, Toulouse, France.
INSERM U1027, Toulouse, Paul Sabatier University, France
E-mail address: cathy.nabet@univ-tlse3.fr

6: Michel Sixou, PhD, DDS
Paul Sabatier University, Dental Faculty, Department of Epidemiology and Public Health, Toulouse University Hospital, Toulouse, France.
E-mail address: michel.sixou@univ-tlse3.fr

7: Jean-Noel Vergnes, PhD, DDS
Paul Sabatier University, Dental Faculty, Department of Epidemiology and Public Health, Toulouse University Hospital, Toulouse, France.
&
Division of Oral Health and Society, Faculty of dentistry, McGill University, Montreal, Quebec, Canada.
E-mail address: jn.vergnes@mcgill.ca

Keywords

Periodontal Diseases; Periodontal Medicine; Periodontitis; Systematic Review; Clinical Trials

Conflict of Interest and Source of Funding

This study was supported by funding from the Midi-Pyrenees region, Paul Sabatier University and the research platform of Toulouse Dental Faculty. We declare that we have nothing to disclose.

Abstract

Aim: The primary aim of the study was to systematically map registration records on periodontal medicine in clinical trial registers. The secondary aim was to assess the evolution of periodontal medicine in clinical periodontal research as a whole.

Material and methods: We searched all registration records related to periodontology in the World Health Organization International Clinical Trials Registry Platform. For registration records classified in the field of periodontal medicine, we assigned the 2015 MeSH® term for the most precisely corresponding systemic condition.

Results: Fifty-seven systemic conditions have been hypothesized to be linked with periodontal diseases, covering nearly 2% of the diseases indexed in MeSH. In addition to diabetes, cardiovascular disease or preterm birth, other systemic conditions have been the subject of registration records, such as anaemia, liver diseases, dyspepsia or ankylosing spondylitis. A trend towards increasing diversification of systemic conditions has appeared over time. About a third of registration records in clinical periodontal research deals with periodontal medicine.

Conclusions: Periodontal medicine now constitutes an important part of clinical periodontal research. Research activity in periodontal medicine has grown continuously since the early 2000's, and exploration of registers gives a useful up-to-date snapshot of this constantly evolving field of research.

Clinical relevance

Scientific rationale for study: "Periodontal medicine" establishes a two-way relationship between periodontal diseases and overall health. There has not yet been any attempt to systematically map and catalogue the systemic conditions potentially associated with periodontal diseases.
Principal findings: Fifty-seven systemic conditions have been hypothesized to be linked with periodontal diseases, which suggests the possibility of thinking in terms of common pathophysiological processes. Research in periodontal medicine is a very active field in current periodontal research.
Practical implications: Knowledge in periodontal medicine would guide practitioners towards both evidence-based and patient-centred approaches when treating patients with systemic conditions.

Introduction

Since the 1990s, the periodontal community has increasingly pointed toward links between periodontal health and systemic conditions, leading to the practical "periodontal medicine" concept. Periodontal medicine establishes a two-way relationship between periodontal diseases and overall health (Garcia et al., 2001, Williams and Offenbacher, 2000). The idea that periodontal diagnosis and treatment could not only preserve the function and aesthetics of the natural dentition but also prevent untoward effects on a patient's overall health (Matthews, 2000), has rapidly spread in the professional (Paquette et al., 2015) and academic (Wilder et al., 2009) communities. Several pathophysiological mechanisms have been put forward, such as bacteraemia, endotoxaemia, and release of inflammatory mediators from periodontal tissues or from an induced acute-phase response (Hajishengallis, 2015, Schenkein and Loos, 2013, Van Dyke and van Winkelhoff, 2013).

The European Federation of Periodontology and the American Academy of Periodontology organized a "workshop on periodontitis and systemic diseases", and reviewed many systemic conditions in association with periodontal diseases. Peer-reviewed reports from this workshop were published in the 2013 supplements of the Journal of Clinical Periodontology (volume 40 Suppl. 14) and the Journal of Periodontology (volume 84 Suppl. 4). These supplements highlighted three major topics that have been extensively investigated in the literature since the 1990s: the links of periodontal diseases with 1) cardiovascular diseases (D'Aiuto et al., 2013, Dietrich et al., 2013, Tonetti and Van Dyke, 2013), 2) diabetes (Borgnakke et al., 2013, Chapple and Genco, 2013, Engebretson and Kocher, 2013), and 3) adverse pregnancy outcomes (Ide and Papapanou, 2013, Michalowicz et al., 2013, Sanz and Kornman, 2013). A fourth topic (Linden et al., 2013, Linden and Herzberg, 2013) concerned associations reported between periodontitis and cancer, chronic kidney diseases, chronic obstructive pulmonary disease, mild cognitive impairment, metabolic syndrome, obesity, or rheumatoid arthritis. Other narrative reviews such as that by (Gulati et al., 2013), have also reported, but with relatively scant evidence, possible links between periodontal diseases and erectile dysfunction, gastrointestinal disease, osteoporosis, pneumonia or prostatitis. Virtually all original studies describing these associations have concluded on the probable existence of confounding factors (not always modifiable), that could explain the co-occurrence of adverse conditions between oral and overall health (Dietrich and Garcia, 2005, Kolk et al., 2012).

Thus, well-designed observational studies are necessary to better understand these associations (Dietrich and Garcia, 2005, Linden et al., 2013). Working group 4 of the workshop on periodontitis and systemic diseases concluded that the field of periodontal medicine was still wide open, with gaps that must be addressed. Globally, the periodontal community agrees that the causation/severity effect of periodontal diseases on cardiovascular diseases, diabetes and adverse pregnancy outcomes needs to be further investigated (Linden and Herzberg, 2013), as shown by recent non-significant results of large, multicentre, randomized controlled trials (RCTs) or meta-analyses (Engebretson et al., 2013, Li et al., 2015, Offenbacher et al., 2009). RCTs are also desirable to develop more precise clinical guidelines for the periodontal management of patients with systemic conditions (Borgnakke et al., 2013).

During the 1990s, clinical trial registers began to be strongly promoted in biomedical research, with the aim of increasing transparency in clinical research (Moher, 1995). In 2005, the International Committee of Medical Journal Editors (ICMJE) recommended that authors of RCT publications register their studies in a publicly accessible trial register (De Angelis et al. 2004). As the start of clinical trial registers coincided with the development of periodontal medicine at the end of the 1990's, the registers provide a good opportunity to map research activity in periodontal medicine. As far as we know, there has not yet been any attempt to systematically map and tabulate the systemic conditions thought to be potentially associated with periodontal diseases. Moreover, optimizing future research resources requires an objective identification of gaps and emerging trends. In particular, systemic conditions related to periodontal diseases could be catalogued in order to describe patterns of actual research activity, potentially revealing common pathophysiological hypotheses (Linden and Herzberg, 2013).

The primary aim of this study was to describe the systemic conditions that have been hypothesized to be related to periodontal diseases, by systematically mapping clinical trial registers. The secondary aim was to assess the evolution of periodontal medicine in clinical periodontal research as a whole.

Material and methods

We followed the PRISMA statement (Moher et al., 2009) for conduct and reporting of systematic reviews.

Search strategy and exclusion criteria

We searched in the World Health Organization International Clinical Trials Registry Platform (ICTRP, www.who.int/trialsearch/). This platform is an umbrella group of 16 registries worldwide, including ClinicalTrials.gov. The latter is under the responsibility of the U.S. government and, at the time of writing, contains more than 200.000 studies from 190 countries, of which 40% originated from the USA. All of these registries must "be accessible to the public at no charge, be open to all prospective registrants, be managed by a not-for-profit organization, have a mechanism to ensure the validity of the registration data, and be electronically searchable" (ICJME 2014).

We employed an iterative search strategy in order to adopt a systematic approach for knowledge discovery. This iterative process took shape as the process evolved (Finfgeld-Connett and Johnson, 2013). The first iteration was "periodontal", and the final iteration (i.e. the final search strategy) was "periodont* OR gingiv* OR *implantitis". The search was performed in each register, without a time limit. The last search was conducted for all registers on 27 May 2015.

Registration records not dealing directly with clinical periodontal research (e.g. on orthodontic research, prosthodontic research, non-periodontal surgery research, and research on apical periodontitis) were excluded from the analysis.

Categorization of registration records

A trial was defined as "any research project that prospectively assigns people or a group of people to an intervention, with our without concurrent comparison or control groups, to study the cause-and-effect relationship between a health-related intervention and a health outcome" (ICJME 2014). Clinical and/or biological outcomes were considered. Based on record contents, we classified as "observational studies" any registration records of cross-sectional,

case-control, cohort or diagnostic studies (since most trial registers are open to registration of observational studies (Loder et al., 2010)).

Among the registration records included, we defined four categories of studies:

i. *Category A: Periodontal medicine - Periodontal intervention to improve (or prevent) a systemic condition*. The intervention could be any type of periodontal treatment. The systemic condition could be any type of adverse condition (or a special physiological process), excluding any type of stomatognathic disease.

ii. *Category B: Periodontal medicine – Intervention for a better understanding of the links between oral and overall health (B1) OR Observational study of a possible link between periodontal disease and a systemic condition (B2).*
Category B1 included any trial adding to knowledge in periodontal medicine without fulfilling the requirements for Category A.
Category B2 included any observational study focusing on both periodontal health and any type of systemic condition (excluding any type of stomatognathic disease).

iii. *Category C: Periodontal intervention to improve oral health.* The outcome was related to periodontal health, or could also be related - but limited - to any type of other stomatognathic diseases.

iv. *Category D: observational studies in periodontal research, without systemic assessment*.

Thus we defined records in the field of periodontal medicine as those registered in categories A+B1+B2. In contrast, we defined as "periodontal dentistry" the area of research dealing with periodontal health at mouth level only (categories C+D). Observational studies were B2+D. No cross-category classification was allowed for a single registration. In case of multiple categorization, the record was placed in the highest-order category (i.e. A>B1>B2>C>D).

Description of systemic conditions

We used the 2015 Medical Subject Headings (MeSH®), from the U.S. National Library of Medicine, as already done in other mapping research (Shen et al., 2011, Komenda et al., 2015). MeSH® is a controlled vocabulary thesaurus used for indexing articles for PubMed (http://www.ncbi.nlm.nih.gov/mesh). It has 16 main branches [A to N + V + Z] and its hierarchical classification leads to narrower terms (taxa). For example, within the [Diseases - C] branch, there are 26 main sub-branches representing narrower categories of disease (e.g.

[C02] Virus diseases, [C07] Stomatognathic Diseases, or [C14] Cardiovascular Diseases). We considered as a potential systemic condition any MeSH term (taxon) that was not referenced in [C07]. Although the main term "Stomatognathic disease [C07]" is not commonly used in scientific and professional literature, sub-branches contain all common oral diseases, such as Periodontitis [C07.465.714.533] or Gingivitis [C07.465.714.258.480].

As the same MeSH terms are often found in several sub-branches, we drew up a simplified, tailored thesaurus in order to avoid redundancy in the mapping process (the one exception being for kidney diseases, where no distinction could be made between male and female disorders). We also considered the [Phenomena and Processes - G] branch, because it contained some possible relevant health conditions, such as pregnancy or menopause. The simplified thesaurus is provided in Supplementary File S1.

Data processing, extraction and analyses

All registration records were exported from ICTRP into XML format, then parsed into a local database. A script was developed to minimize errors during the screening process and to computerize data for further analyses. The script was based on a Perl Web Dancer 1.3 framework (http://perldancer.org/).

Evaluators (PM, JNV) classified each registration record in Category A, B1, B2, C or D. Any discrepancies were resolved through discussion and consensus. The two evaluators (PM, JNV) also extracted the following items: year of study start, sample size, country of the main centre, and register. Multiple records about the same study were identified, and only the main registration (put forward by the ICTRP) was taken into account to avoid duplicate information.

For registration records in the field of periodontal medicine (Categories A, B1 and B2), the two evaluators (PM, JNV) assigned the most precise term (taxon) of the MeSH classification, corresponding to the systemic condition described in the study. In cases with several distinct systemic conditions within a registration record, multiple assignments were allowed. The resulting tree was built up with all assigned MeSH terms, along with their proximal ramifications, since the MeSH classification is hierarchical.

Median and interquartile ranges were provided for continuous characteristics of studies while frequencies and percentages were provided for categorical characteristics, as described by (Califf et al., 2012).

Data visualization

Chord diagrams or connectograms (Krzywinski et al., 2009) were used to link the proportion of registrations by [Diseases-C] sub-branches, to 1) their categories (classified as described above as A, B1, B2, C or D) and 2) the start years (or anticipated start years) of the studies. They were composed of two rings. The inner ring included categorical segments, the size of which was proportional to the corresponding number of registration records. The outer ring represented the corresponding relative percentage of each sub-part of the segments. Coloured ribbons made connections between sub-parts of different categorical segments (Krzywinski et al., 2009). An annotated figure is provided in Supplementary file S2 to help the reader's interpretation.

From the resulting MeSH terms tree, the visual mapping of systemic conditions potentially related to periodontal diseases was represented using a circular phylogenetic-like tree. After adequate formatting (nexus format), the phylogenetic-like tree was obtained with the tool available at http://itol.embl.de/ (Letunic and Bork, 2007). Lines from the centre to the periphery of the circle represent the hierarchical tree structure of the MeSH®. At the end of each taxon, we added a two-coloured rectangular bar, of length proportional to the relative number of registration records in categories A (red) and B1+B2 (grey).

Results

A total of 966 registration records were retrieved from ICTRP, of which 144 were excluded because they did not deal directly with periodontal research (full list available in Supplementary File S3).

Descriptive analysis of registration records

Among the 822 selected records, there were 129 trials classified in Category A, 36 trials in Category B1 and 77 in Category B2. Thus there were 242 registration records (29.5%) dealing with periodontal medicine.

Complementarily, there were 524 trials classified in Category C and 56 studies in Category D, for a majority of 580 (70.5%) records dealing with periodontal dentistry. The term "*implantitis" was found in 21 titles of registration records, and none of these records were classified in the periodontal medicine field of research. There were 133 (16.2%) observational studies registered.

Some characteristics of registration records are presented in Table 1, and the list of all registration records by categories is available in Supplementary File S3. More than half the records were registered in the American clinicaltrials.gov register. Registration records were mainly from Asia, Europe and North America: 51.4%, 23.9% and 14.8% for periodontal dentistry, 42.5%, 20.8% and 18.1% for periodontal medicine, respectively.

Systemic conditions, once labelled as MeSH terms, were assigned into the narrowest taxon in the simplified tree (available in Supplementary File S1).

Figure 1 represents the connections between Categories A, B1+B2, C, D and MeSH sub-branches from [C] and [G]. In this figure, registration records within the scope of periodontal dentistry are linked to the [C07] Stomatognathic Diseases branch.

Systemic conditions in periodontal medicine

Periodontal diseases were found to be linked to 17 of the 26 main sub-branches of the Diseases [C] branch, i.e. all [C] sub-branches except Parasitic Diseases [C03], Stomatognathic Disease [C07] (by voluntary design), Otorhinolaryngologic Diseases [C09], Congenital, Hereditary, and Neonatal Diseases and Abnormalities [C16], Disorders of Environmental Origin [C21], Animal Diseases [C22], Occupational Diseases [C24], Chemically-Induced Disorders [C25], and Wounds and Injuries [C26]. Exploding the tree from each of the MeSH terms involved gave a total of 129 MeSH terms that have been potentially associated with periodontal diseases. This constitutes 1.95% of total [C] and [G] MeSH terms. Only two studies had multiple categories: JPRN-UMIN000008582 (Diabetes Mellitus and Arteriosclerosis) and JPRN-UMIN000013751 (Diabetes Mellitus and Rheumatoid Arthritis).

The super-tree in Figure 2 shows the 57 systemic conditions that have appeared in the tabulation to be related to periodontal diseases. We could distinguish between major topics (more than 30 records, i.e. diabetes, inflammation and cardiovascular diseases), intermediate topics (between 5 and 30 records, e.g. pregnancy complications, rheumatoid arthritis,

respiratory tract diseases, or kidney diseases) and minor topics (fewer than 5 records, e.g. neoplasms, gastroesophageal reflux, anaemia, liver diseases, dyspepsia, ankylosing spondylitis or male infertility). Minor topics were often designed as observational studies (Category B2). A complete list of these systemic conditions along with the corresponding numbers of registration records in Categories A, B1 and B2, is provided in Supplementary File S4.

Evolution of registration records in periodontal research

Figure 3 shows that there is a clear trend towards growing registration of studies, according to their starting years from the late 1990's to 2014 and beyond. Relative to this growth, there is no major difference between periodontal medicine and periodontal dentistry: between 1998 and 2013, the average annual growth rate of records was 39% for periodontal medicine (A+B1+B2) and 35% for periodontal dentistry (C+D).

Figure 4 shows a temporal evolution of topics dealing with periodontal medicine, with the start dates of the studies. Diabetes, systemic inflammation, pregnancy complications and cardiovascular diseases have always been the main topics in periodontal medicine. A snowball effect can also be detected: minor topics in one period are likely to become intermediate topics later (e.g. musculoskeletal diseases, or male urogenital diseases). Finally, there appears to be a trend towards increasing diversification in the registration records.

Discussion

Periodontal medicine now constitutes an important part of clinical periodontal research, continuously growing since the early 2000's. Our analysis estimates that at least 1.95% of the MeSH terms (within the Disease and the Phenomena and Processes sub-branches) have now been hypothesized to be linked to periodontal diseases.

We showed that putative systemic conditions associated with periodontal diseases are diversifying over time. While research on cardiovascular diseases, diabetes or pregnancy complications is still very active, more and more systemic conditions are suspected to be linked with periodontal diseases and our systematic mapping found anaemia, liver diseases, dyspepsia or ankylosing spondylitis as examples of emerging topics in periodontal medicine.

Our results also indicate that periodontal medicine constitutes an important part of periodontal research. About a third of periodontal studies registered in trials registers deals with possible links between periodontal health and overall health. Although some researchers argue that periodontal medicine has been a “driving force for a considerable downscaling of research into periodontitis in its own right” (Baelum and Lopez, 2013), that is not what we found in this analysis of clinical trial registers. Considering periodontal medicine as the new paradigm of periodontology, Baelum and Lopez (2013) underestimate the large amount of periodontal research still conducted with the aim of improving prophylactic, diagnostic, therapeutic, or rehabilitative measures in periodontology. Though it is likely that periodontal medicine could have increased the funding opportunities for periodontal research (Baelum and Lopez, 2013), there is no evidence whatsoever that it has a detrimental effect on what we call "periodontal dentistry".

This study has several limitations that should be considered. One major limitation is that all registration records were considered as having the same level of methodological quality. There is no consensus on how to assess the quality of registration records. A recent survey of records taken from ICTRP showed that there are still important problems with completeness of registration records (Viergever et al., 2014). This could have led to some inaccuracies in our systematic mapping process. For example, only the main centre of the trials was extracted. ClinicalTrials.gov offers the possibility to also extract all the centres of a multi-centre study, and the mean number of centres per study may be an interesting parameter to investigate in order to explore worldwide scientific interactions (Cheng et al., 2012). However, we have shown that the number of trial registrations is growing over time, which may reflect a wider variety of therapeutic interventions provided by professionals (Nishimura et al., 2002). This trend may be analysed as beneficial for patients but may also reflect a lack of consensus on efficacy and optimal periodontal care (Niederman et al., 2002). Another limitation is that only half of the existing biomedical journals have adhered to the ICMJE requirement in their instructions to authors (Hooft et al., 2014). In dentistry, instructions to authors of numerous journals require (e.g. J Dent Res, J Periodontol) or strongly encourage (e.g. J Clin Periodontol, Clin Oral Implan Res) authors who submit manuscripts reporting on a trial to register their trial. However it has recently been shown that only one-quarter of RCTs published in oral health journals are publicly registered (Smail-Faugeron et al., 2015). The number of systemic conditions that have been associated with periodontal diseases in the periodontal literature is likely to have been underestimated in our analysis. For example, we

did not find any registration records on the topic of prostatitis and periodontal disease, yet it has already been described in the literature (Joshi et al., 2010). One explanation is that our analysis was not designed to assess the overall literature but only trial registers. Periodontal research also requires preclinical models for preliminary safety and proof-of-concept before translation to humans (Sculean et al., 2015). There is currently no specific initiative for the registration of observational studies (Guyatt et al., 2011), laboratory research and/or animal research. We identified less than 20% of observational studies, and no *in-vitro* or animal studies. A systematic mapping of the whole PubMed database would give a sharper picture of actual periodontal research and a representation of the interventional/observational ratio, allowing the discovery of pathophysiological links between periodontitis and systemic diseases through animal models (Hajishengallis et al., 2015). Comparison between animal and human studies should also be investigated (Perel et al., 2007).

Finally, our study could be criticized for taking only registration records into account, which have not been peer-reviewed. Analyses of published studies take advantage of the scientific peer-review process but publication is often delayed and negative trials are less likely to be published (Dickersin and Rennie, 2012). Full-text final articles would give the opportunity to map the time needed between registration, start date, and the end of the study. In spite of these limitations, exploration of registers gives a more up-to-date snapshot of the constantly evolving field of periodontal medicine.

It is also important to be cautious regarding inferences to be drawn and interpretations to be made from our analysis. First, we strongly emphasize that the fact of systemic conditions being associated with periodontal diseases in registration records does not mean that significant links were found between periodontal status and systemic conditions. This systematic mapping was simply aimed to tabulate topics of interest in periodontal medicine. Second, we emphasize the danger that the dental profession could press to justify the importance of dental treatment by highlighting its impact on various systemic conditions (Tenenbaum et al., 2007), such as the ones described in this study, although the causal relationships are still rarely proved. Such behaviour would undermine the credibility of scientific research and academic recommendations. Third, it is far from our intention to spread the idea that oral health and systemic health could be two different entities. As pointed out previously, talking about the “oral-systemic health connection” could unintentionally separate the mouth from the rest of the body (Nogueira-Filho and Tenenbaum, 2011). In fact,

we acknowledge that the overall health of patients is of tremendous importance for dentists seeking to achieve genuine whole-patient care.

From this study, several perspectives of research in periodontal medicine can be outlined. More than ever, well-designed observational studies are needed to better understand associations between periodontal diseases and systemic diseases (Dietrich and Garcia, 2005), since observational studies are often the only feasible method for studying most questions concerning risk. Although we analysed data from trial registers, we found a substantial proportion of the registered trials to be observational studies (16%). This was consistent with other fields of biomedical research, such as surgical oncology, where 15% of registration records in ClinicalTrials.gov were observational studies (Menezes et al., 2013).

Regarding the main topics (i.e. cardiovascular diseases, adverse pregnancy outcomes and diabetes), published RCTs have produced discordant results, but there is still a need to assess different modalities of periodontal treatment for individuals with systemic conditions (Vergnes, 2014).

Although periodontal treatment modalities fell outside the scope of the present work, the screening of trial registers has revealed great diversity in this field (Bader, 2010, Drisko, 2001). ~~Evidence about which types of treatment may be helpful and which may be harmful, in whom, and why, is needed to guide practice and policy (Vohra and Boon, 2015).~~ We did not notice alternative methods of interventional research, such as n-of-1 trials (Lillie et al., 2011), a methodology that has never been investigated in periodontal research as far as we know.

Finally, we also noted a lack of patient-centred outcomes in RCTs, while this concept is of fundamental importance considering that whole-person care is the cornerstone of periodontal medicine.

Perhaps the most exciting and challenging perspective in periodontal medicine would be the diversification of meta-research. The current prolific production of scientific information and the burst of metadata make systematic work very necessary to classify and stratify all the accumulated data and knowledge (Altman et al., 2008). In addition to traditional systematic reviews (essentially based on deductive approaches), other validated approaches to systematic reviewing are hardly explored in periodontal medicine. For example, realist syntheses (Rycroft-Malone et al., 2012), although time-consuming and human resource intensive, could give opportunities to develop more pragmatically insightful conclusions in periodontal medicine, while taking results from both fundamental sciences and clinical studies into account. Data mining and Knowledge Discovery in Databases (KDD) on periodontal research

(Hettne et al., 2007), as initiated in the present analysis, would highlight the necessity for new research directions (Fayyad U et al., 1996, Piatetsky-Shapiro and Frawley, 1991), and would also identify gaps in research. The combination of bioinformatics tools and medical data would result in significant advances in the understanding of pathophysiology and individual susceptibility (Hettne et al., 2007). A first step in this direction could be the addition of "Periodontal Medicine" as a new taxon in the MeSH classification, for example as a sub-branch of Periodontics, under Health Occupations [H02], Dentistry [H02.163], Specialties, Dental [H02.163.876], Periodontics [H02.163.876.623].

In conclusion, analyses of trial registers showed that research in periodontal medicine is a very active field in periodontology. Fifty-seven systemic conditions have been hypothesized to be linked with periodontal diseases, which suggests the possibility of thinking in terms of common pathophysiological processes. Knowledge in periodontal medicine would guide practitioners towards both evidence-based and patient-centred approaches when treating patients with systemic conditions.

References

Altman, R. B., Balling, R., Brinkley, J. F., Coiera, E., Consorti, F., Dhansay, M. A., Geissbuhler, A., Hersh, W., Kwankam, S. Y., Lorenzi, N. M., Martin-Sanchez, F., Mihalas, G. I., Shahar, Y., Takabayashi, K. & Wiederhold, G. (2008) Commentaries on "Informatics and medicine: from molecules to populations". *Methods of Information in Medicine* **47,** 296-317.

Bader, H. I. (2010) Adjunctive periodontal therapy: a review of current techniques. *Dentistry Today* **29,** 94-96, 98; quiz 98, 103.

Baelum, V. & Lopez, R. (2013) Periodontal disease epidemiology - learned and unlearned? *Periodontology 2000* **62,** 37-58. doi:10.1111/j.1600-0757.2012.00449.x.

Borgnakke, W. S., Ylostalo, P. V., Taylor, G. W. & Genco, R. J. (2013) Effect of periodontal disease on diabetes: systematic review of epidemiologic observational evidence. *Journal of Clinical Periodontology* **40 Suppl 14,** S135-152. doi:10.1111/jcpe.12080.

Califf, R. M., Zarin, D. A., Kramer, J. M., Sherman, R. E., Aberle, L. H. & Tasneem, A. (2012) Characteristics of clinical trials registered in ClinicalTrials.gov, 2007-2010. *JAMA* **307,** 1838-1847. doi:10.1001/jama.2012.3424.

Chapple, I. L. & Genco, R. (2013) Diabetes and periodontal diseases: consensus report of the Joint EFP/AAP Workshop on Periodontitis and Systemic Diseases. *Journal of Clinical Periodontology* **40 Suppl 14,** S106-112. doi:10.1111/jcpe.12077.

Cheng, S. K., Hirsch, B. R., Califf, R. M., Tasneem, A., Schulman, K. A., Abernethy, A. P. & Dilts, D. M. (2012) Geographic and network analysis of oncology trials: Portfolio assessment of ClinicalTrials.gov. In: *ASCO Annual Meeting*.

D'Aiuto, F., Orlandi, M. & Gunsolley, J. C. (2013) Evidence that periodontal treatment improves biomarkers and CVD outcomes. *Journal of Clinical Periodontology* **40 Suppl 14,** S85-105. doi:10.1111/jcpe.12061.

Dickersin, K. & Rennie, D. (2012) The evolution of trial registries and their use to assess the clinical trial enterprise. *JAMA* **307,** 1861-1864. doi:10.1001/jama.2012.4230.

Dietrich, T. & Garcia, R. I. (2005) Associations between periodontal disease and systemic disease: evaluating the strength of the evidence. *Journal of Periodontology* **76,** 2175-2184. doi:10.1902/jop.2005.76.11-S.2175.

Dietrich, T., Sharma, P., Walter, C., Weston, P. & Beck, J. (2013) The epidemiological evidence behind the association between periodontitis and incident atherosclerotic cardiovascular disease. *Journal of Clinical Periodontology* **40 Suppl 14,** S70-84. doi:10.1111/jcpe.12062.

Drisko, C. H. (2001) Nonsurgical periodontal therapy. *Periodontology 2000* **25,** 77-88.

Engebretson, S. & Kocher, T. (2013) Evidence that periodontal treatment improves diabetes outcomes: a systematic review and meta-analysis. *Journal of Clinical Periodontology* **40 Suppl 14,** S153-163. doi:10.1111/jcpe.12084.

Engebretson, S. P., Hyman, L. G., Michalowicz, B. S., Schoenfeld, E. R., Gelato, M. C., Hou, W., Seaquist, E. R., Reddy, M. S., Lewis, C. E., Oates, T. W., Tripathy, D., Katancik, J. A., Orlander, P. R., Paquette, D. W., Hanson, N. Q. & Tsai, M. Y. (2013) The effect

of nonsurgical periodontal therapy on hemoglobin A1c levels in persons with type 2 diabetes and chronic periodontitis: a randomized clinical trial. *JAMA* **310,** 2523-2532. doi:10.1001/jama.2013.282431.

Fayyad U, Piatetsky-Shapiro G & P, S. (1996) From Data Mining to Knowledge Discovery in Databases. *AI Magazine* **17,** 37-54. doi:http://dx.doi.org/10.1609/aimag.v17i3.1230.

Finfgeld-Connett, D. & Johnson, E. D. (2013) Literature search strategies for conducting knowledge-building and theory-generating qualitative systematic reviews. *Journal of Advanced Nursing* **69,** 194-204. doi:10.1111/j.1365-2648.2012.06037.x.

Garcia, R. I., Henshaw, M. M. & Krall, E. A. (2001) Relationship between periodontal disease and systemic health. *Periodontology 2000* **25,** 21-36.

Gulati, M., Anand, V., Jain, N., Anand, B., Bahuguna, R., Govila, V. & Rastogi, P. (2013) Essentials of periodontal medicine in preventive medicine. *International Journal of Preventive Medicine* **4,** 988-994.

Guyatt, G. H., Oxman, A. D., Montori, V., Vist, G., Kunz, R., Brozek, J., Alonso-Coello, P., Djulbegovic, B., Atkins, D., Falck-Ytter, Y., Williams, J. W., Jr., Meerpohl, J., Norris, S. L., Akl, E. A. & Schunemann, H. J. (2011) GRADE guidelines: 5. Rating the quality of evidence--publication bias. *Journal of Clinical Epidemiology* **64,** 1277-1282. doi:10.1016/j.jclinepi.2011.01.011.

Hajishengallis, G. (2015) Periodontitis: from microbial immune subversion to systemic inflammation. *Nature Reviews. Immunology* **15,** 30-44. doi:10.1038/nri3785.

Hajishengallis, G., Lamont, R. J. & Graves, D. T. (2015) The enduring importance of animal models in understanding periodontal disease. *Virulence* **6,** 229-235. doi:10.4161/21505594.2014.990806.

Hettne, K. M., Weeber, M., Laine, M. L., ten Cate, H., Boyer, S., Kors, J. A. & Loos, B. G. (2007) Automatic mining of the literature to generate new hypotheses for the possible link between periodontitis and atherosclerosis: lipopolysaccharide as a case study. *Journal of Clinical Periodontology* **34,** 1016-1024. doi:10.1111/j.1600-051X.2007.01152.x.

Hooft, L., Korevaar, D. A., Molenaar, N., Bossuyt, P. M. & Scholten, R. J. (2014) Endorsement of ICMJE's Clinical Trial Registration Policy: a survey among journal editors. *The Netherlands Journal of Medicine* **72,** 349-355.

Ide, M. & Papapanou, P. N. (2013) Epidemiology of association between maternal periodontal disease and adverse pregnancy outcomes--systematic review. *Journal of Clinical Periodontology* **40 Suppl 14,** S181-194. doi:10.1111/jcpe.12063.

Joshi, N., Bissada, N. F., Bodner, D., Maclennan, G. T., Narendran, S., Jurevic, R. & Skillicorn, R. (2010) Association between periodontal disease and prostate-specific antigen levels in chronic prostatitis patients. *Journal of Periodontology* **81,** 864-869. doi:10.1902/jop.2010.090646.

Kolk, A., Handschel, J., Drescher, W., Rothamel, D., Kloss, F., Blessmann, M., Heiland, M., Wolff, K. D. & Smeets, R. (2012) Current trends and future perspectives of bone substitute materials - from space holders to innovative biomaterials. *Journal of Craniomaxillofacial Surgery* **40,** 706-718. doi:10.1016/j.jcms.2012.01.002.

Komenda, M., Schwarz, D., Svancara, J., Vaitsis, C., Zary, N. & Dusek, L. (2015) Practical use of medical terminology in curriculum mapping. *Computers in Biology and Medicine* **63,** 74-82. doi:10.1016/j.compbiomed.2015.05.006.

Krzywinski, M., Schein, J., Birol, I., Connors, J., Gascoyne, R., Horsman, D., Jones, S. J. & Marra, M. A. (2009) Circos: an information aesthetic for comparative genomics. *Genome Research* **19,** 1639-1645. doi:10.1101/gr.092759.109.

Letunic, I. & Bork, P. (2007) Interactive Tree Of Life (iTOL): an online tool for phylogenetic tree display and annotation. *Bioinformatics* **23,** 127-128.

Li, Q., Hao, S., Fang, J., Xie, J., Kong, X. H. & Yang, J. X. (2015) Effect of non-surgical periodontal treatment on glycemic control of patients with diabetes: a meta-analysis of randomized controlled trials. *Trials* **16,** 291. doi:10.1186/s13063-015-0810-2.

Lillie, E. O., Patay, B., Diamant, J., Issell, B., Topol, E. J. & Schork, N. J. (2011) The n-of-1 clinical trial: the ultimate strategy for individualizing medicine? *Personalized Medicine* **8,** 161-173. doi:10.2217/pme.11.7.

Linden, G. J. & Herzberg, M. C. (2013) Periodontitis and systemic diseases: a record of discussions of working group 4 of the Joint EFP/AAP Workshop on Periodontitis and Systemic Diseases. *Journal of Clinical Periodontology* **40 Suppl 14,** S20-23. doi:10.1111/jcpe.12091.

Linden, G. J., Lyons, A. & Scannapieco, F. A. (2013) Periodontal systemic associations: review of the evidence. *Journal of Clinical Periodontology* **40 Suppl 14,** S8-19. doi:10.1111/jcpe.12064.

Loder, E., Groves, T. & Macauley, D. (2010) Registration of observational studies. *British Medical Journal* **340,** c950. doi:10.1136/bmj.c950.

Matthews, D. C. (2000) Periodontal medicine: a new paradigm. *Journal of Canadian Dental Association* **66,** 488-491.

Menezes, A. S., Barnes, A., Scheer, A. S., Martel, G., Moloo, H., Boushey, R. P., Sabri, E. & Auer, R. C. (2013) Clinical research in surgical oncology: an analysis of ClinicalTrials.gov. *Annals of Surgical Oncology* **20,** 3725-3731. doi:10.1245/s10434-013-3054-y.

Michalowicz, B. S., Gustafsson, A., Thumbigere-Math, V. & Buhlin, K. (2013) The effects of periodontal treatment on pregnancy outcomes. *Journal of Clinical Periodontology* **40 Suppl 14,** S195-208. doi:10.1111/jcpe.12081.

Moher, D. (1995) Facilitating clinical research in the 1990s and beyond: challenges facing clinical trial registers. *Fundamental & Clinical Pharmacology* **9,** 381-383.

Moher, D., Liberati, A., Tetzlaff, J. & Altman, D. G. (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Journal of Clinical Epidemiology* **62,** 1006-1012. doi:10.1016/j.jclinepi.2009.06.005.

Niederman, R., Chen, L., Murzyn, L. & Conway, S. (2002) Benchmarking the dental randomised controlled literature on MEDLINE. *Evidence-Based Dentistry* **3,** 5-9.

Nishimura, K., Rasool, F., Ferguson, M. B., Sobel, M. & Niederman, R. (2002) Benchmarking the clinical prosthetic dental literature on MEDLINE. *The Journal of Prosthetic Dentistry* **88,** 533-541. doi:10.1067/mpr.2002.129301.

Nogueira-Filho, G. & Tenenbaum, H. C. (2011) So why do we call it the oral-systemic health connection? *Journal of the Canadian Dental Association* **77,** b36.

Offenbacher, S., Beck, J. D., Jared, H. L., Mauriello, S. M., Mendoza, L. C., Couper, D. J., Stewart, D. D., Murtha, A. P., Cochran, D. L., Dudley, D. J., Reddy, M. S., Geurs, N. C. & Hauth, J. C. (2009) Effects of periodontal therapy on rate of preterm delivery: a randomized controlled trial. *Obstetrics and Gynecology* **114,** 551-559. doi:10.1097/AOG.0b013e3181b1341f.

Paquette, D. W., Bell, K. P., Phillips, C., Offenbacher, S. & Wilder, R. S. (2015) Dentists' knowledge and opinions of oral-systemic disease relationships: relevance to patient care and education. *Journal of Dental Education* **79,** 626-635.

Perel, P., Roberts, I., Sena, E., Wheble, P., Briscoe, C., Sandercock, P., Macleod, M., Mignini, L. E., Jayaram, P. & Khan, K. S. (2007) Comparison of treatment effects between animal experiments and clinical trials: systematic review. *British Medical Journal* **334,** 197. doi:10.1136/bmj.39048.407928.BE.

Piatetsky-Shapiro, G. & Frawley, W. J. (1991) *Knowledge Discovery in Databases*. AAAI/MIT Press.

Rycroft-Malone, J., McCormack, B., Hutchinson, A. M., DeCorby, K., Bucknall, T. K., Kent, B., Schultz, A., Snelgrove-Clarke, E., Stetler, C. B., Titler, M., Wallin, L. & Wilson, V. (2012) Realist synthesis: illustrating the method for implementation research. *Implementation Science* **7,** 33. doi:10.1186/1748-5908-7-33.

Sanz, M. & Kornman, K. (2013) Periodontitis and adverse pregnancy outcomes: consensus report of the Joint EFP/AAP Workshop on Periodontitis and Systemic Diseases. *Journal of Clinical Periodontology* **40 Suppl 14,** S164-169. doi:10.1111/jcpe.12083.

Schenkein, H. A. & Loos, B. G. (2013) Inflammatory mechanisms linking periodontal diseases to cardiovascular diseases. *Journal of Clinical Periodontology* **40 Suppl 14,** S51-69. doi:10.1111/jcpe.12060.

Sculean, A., Chapple, I. L. & Giannobile, W. V. (2015) Wound models for periodontal and bone regeneration: the role of biologic research. *Periodontology 2000* **68,** 7-20. doi:10.1111/prd.12091.

Shen, J., Yao, L., Li, Y., Clarke, M., Gan, Q., Fan, Y., Zhong, D., Gou, Y. & Wang, L. (2011) Visualization studies on evidence-based medicine domain knowledge (series 1): mapping of evidence-based medicine research subjects. *Journal of Evidence Based Medicine* **4,** 73-84. doi:10.1111/j.1756-5391.2011.01126.x.

Smail-Faugeron, V., Fron-Chabouis, H. & Durieux, P. (2015) Clinical trial registration in oral health journals. *Journal of Dental Research* **94,** 8s-13s. doi:10.1177/0022034514552492.

Tenenbaum, H., Matthews, D., Sandor, G. & McCulloch, C. (2007) Oral health-systemic health: what is the true connection? Interviews by Sean McNamara. *Journal of the Canadian Dental Association* **73,** 211-216.

Tonetti, M. S. & Van Dyke, T. E. (2013) Periodontitis and atherosclerotic cardiovascular disease: consensus report of the Joint EFP/AAP Workshop on Periodontitis and Systemic Diseases. *Journal of Clinical Periodontology* **40 Suppl 14,** S24-29. doi:10.1111/jcpe.12089.

Van Dyke, T. E. & van Winkelhoff, A. J. (2013) Infection and inflammatory mechanisms. *Journal of Clinical Periodontology* **40 Suppl 14,** S1-7. doi:10.1111/jcpe.12088.

Vergnes, J. N. (2014) Hemoglobin A1c levels among patients with diabetes receiving nonsurgical periodontal treatment. *JAMA* **311,** 1920-1921. doi:10.1001/jama.2014.2225.

Viergever, R. F., Karam, G., Reis, A. & Ghersi, D. (2014) The quality of registration of clinical trials: still a problem. *PLoS One* **9,** e84727. doi:10.1371/journal.pone.0084727.

Vohra, S. & Boon, H. (2015) Advancing knowledge requires both clinical and basic research. *Focus on Alternative and Complementary Therapies* **20,** 32-33. doi:10.1111/fct.12160.

Wilder, R. S., Iacopino, A. M., Feldman, C. A., Guthmiller, J., Linfante, J., Lavigne, S. & Paquette, D. (2009) Periodontal-systemic disease education in U.S. and Canadian dental schools. *Journal of Dental Education* **73,** 38-52.

Williams, R. C. & Offenbacher, S. (2000) Periodontal medicine: the emergence of a new branch of periodontology. *Periodontology 2000* **23,** 9-12.

Acknowledgements

This study was supported by funding from the Midi-Pyrenees region, Paul Sabatier University and the research platform of the Toulouse Dental Faculty. The authors thank Susan Becker for proofreading. We declare no conflicts of interest.

Figures

Figure 1

Connections between study categories A, B (B1 and B2 combined), C, D, and terms in MeSH sub-branches from [C] and [G].

This chord diagram represents the proportion of studies dealing with each included sub-branch of the MeSH classification "Diseases" [C] and "Phenomena and Processes" [G], by category. Trials concerning periodontal intervention to improve (or prevent) a systemic condition are classified as A; trials concerning interventions for a better understanding of the links between oral and overall health or observational studies between periodontal disease and a systemic condition are classified as B1+B2. A and B1+B2 correspond to periodontal medicine. Periodontal dentistry (the area of research dealing with periodontal health at mouth level only) is interventional (C) or observational (D).

Trial categories (on the right part of the figure) are shaded either dark (A or C) or light grey (B1+B2 or D). On the left part of the figure, each MeSH sub-branch with its colour code is listed in order of descending frequency. The outer ring of the figure indicates the proportion of studies while the inner circle shows their absolute numbers. In each ring, the contribution of included sub-branches of MeSH [C] and [G] in each trial category is coded by coloured segments. These coloured segments are also sorted by frequency.

An annotated figure is provided in Supplementary file S2.

Figure 2

Phylogenetic-like tree of systemic conditions that have been hypothesized in the registers to be related to periodontal diseases.

Lines from the centre to the periphery of the circle represent the hierarchical tree structure of the MeSH classification. At the end of each taxon, a two-coloured rectangular bar has been added, with length proportional to the relative number of registration records in the A (red) and B1+B2 (grey) categories, respectively.

Abbreviations: trials concerning periodontal intervention to improve (or prevent) a systemic condition are classified as A; trials concerning intervention for a better understanding of the links between oral and overall health are classified B1 and observational studies of the link between periodontal disease and a systemic condition are classified B2.

Figure 3

Evolution of registration records in periodontal research according to their starting years from the mid-1990's to 2014 and beyond.

On the Y-axis, the cumulative number of studies is represented for each start year noted on the X-axis. Periodontal medicine is represented in orange (A+B1+B2) and periodontal dentistry in blue (C+D). The mean annual growth rate of records between 1998 and 2013 was 39% for periodontal medicine and 35% for periodontal dentistry.

Trials on periodontal intervention to improve (or prevent) a systemic condition are classified as A; trials on intervention for a better understanding of the links between oral and overall health are classified B1 and observational studies of links between periodontal disease and a systemic condition are classified B2. Periodontal intervention to improve oral health is classified as C; observational studies in periodontal research, without systemic assessment are classified as D.

Figure 4

Connectogram of the temporal evolution of topics dealing with periodontal medicine. This chord diagram represents the proportion of studies dealing with each included sub-branch of the MeSH classification "Diseases" [C] and "Phenomena and Processes" [G], linked to the respective start years of the studies (grouped in 4 periods: 1998-2005, 2006-2008, 2009-2011, 2012 and beyond).

Supplementary Files

Supplementary File S1

Simplified MeSH tree: systemic conditions that have been hypothesized to be linked with periodontal diseases, after labelling as MeSH terms.

Bold: MeSH terms that were selected.
Underlined: Proximal branches of selected MeSH terms.
(~~Crossed out~~): Branches that were removed for data visualization (phylogenetic-like tree and circos connectograms)

Refinement of the resulting MeSH tree before data visualization

As the same MeSH terms are often found in several sub-branches, we drew up a simplified thesaurus in order to avoid redundancy in the mapping process. To gain in readability and to provide a visual map of systemic conditions in periodontal medicine, the resulting MeSH tree was refined in 3 steps.
First, we removed 6 duplicate taxa. As all selected MeSH terms from Endocrine System Diseases [C19] were duplicated in Nutritional and Metabolic Diseases [C18], we kept only [C18] for data visualization. Similarly, as Sjogren's syndrome was already indexed in Skin and Connective Tissue Diseases [C17], we removed Eye Diseases [C11]. Population Characteristics [N01], Genetic Phenomena [G05], Mental Disorders [F03], and Diagnosis [E01] were also removed for duplication reasons.
Second, the MeSH term "Kidney Diseases" was left in both Male Urogenital Diseases [C12] and Female Urogenital Diseases and Pregnancy Complications [C13].

Third, the Phenomena and Processes [G] branch was also selected to refer to 4 MeSH terms (i.e. [G07] Physiological Phenomena, leading to [G07.610.650] Nutritional Status; [G08] Reproductive and Urinary Physiological Phenomena, leading to [G08.686.785.760.769] Pregnancy and [G08.686.157.500] Menopause; and [G12] Immune System Phenomena, leading to [G12.425.901] Transplantation Immunology).
These procedures had no impact on the number of registration records described in the article.

Supplementary File S2

Annotated figure (see Figure 1) for connections between Categories A, B1+B2, C, D and MeSH sub-branches from [C] and [G].

This chord diagram represents the proportion of studies dealing with each included sub-branch of the MeSH classification "Diseases" [C] and "Phenomena and Processes" [G], linked to the respective category of studies. Trials concerning periodontal intervention to improve (or prevent) a systemic condition are classified as A; trials concerning intervention for a better understanding of the links between oral and overall health or observational studies of periodontal disease and a systemic condition are classified as B1+B2. A and B1+B2 correspond to periodontal medicine. Periodontal dentistry (the area of research dealing with periodontal health at mouth level only) is interventional (C) or observational (D).

Supplementary File S3

Exhaustive list of the 966 registration records retrieved from ICTRP with the search strategy, by category (A, B1, B2, C, D, and excluded): registration numbers and titles.

Abbreviations: A - Periodontal intervention to improve (or prevent) a systemic condition; B1 - Intervention for a better understanding of the links between oral and overall health; B2 - Observational study of possible link between periodontal disease and a systemic condition; C - Periodontal intervention to improve oral health; D - observational studies in periodontal research, without systemic assessment.

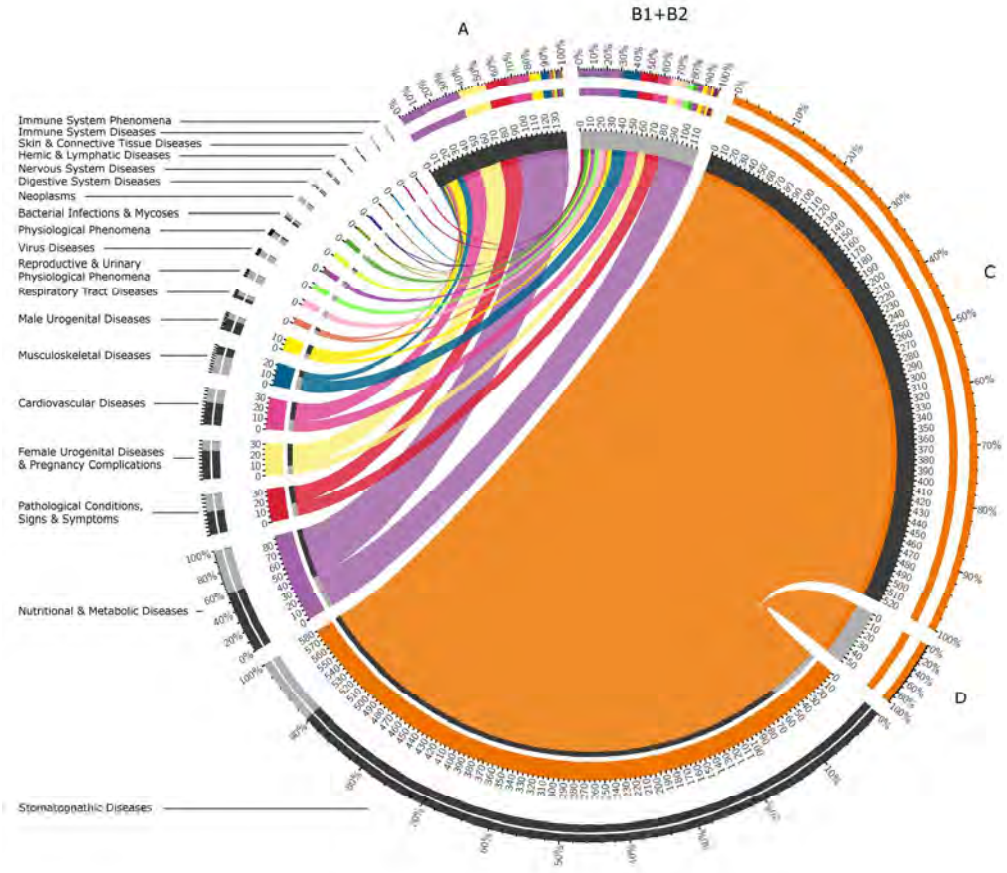
Category A: 129 studies; Category B1: 36 studies; Category B2: 77 studies; Category C: 524 studies; Category D: 56 studies; Excluded: 144 studies

Supplementary File S4

Systemic conditions that have been hypothesized to be linked with periodontal diseases, after labelling as MeSH terms.

A complete list is provided for the 57 systemic conditions that have been hypothesized to be related to periodontal diseases, along with the corresponding number of registration records in Categories A, B1 and B2. Colour code refers to the one chosen in Figures 1, 2 and 4.

Abbreviations: A - Periodontal intervention to improve (or prevent) a systemic condition; B1 - Intervention for a better understanding of the links between oral and overall health; B2 - Observational study of periodontal disease and a systemic condition.

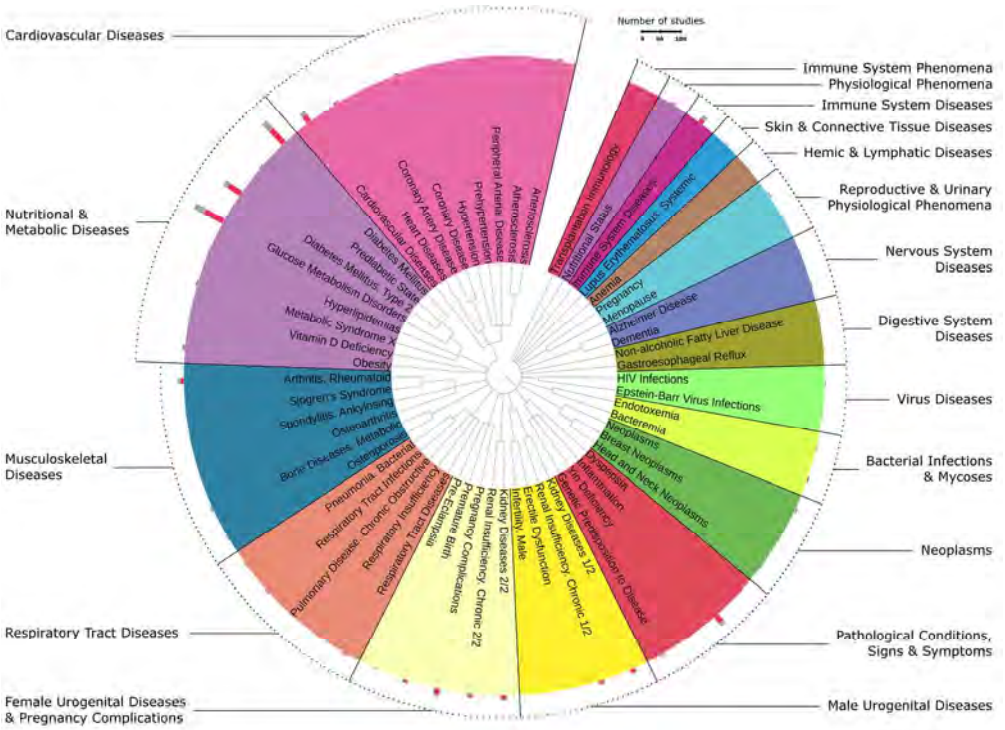


Connections between study categories A, B (B1 and B2 combined), C, D, and terms in MeSH sub-branches from [C] and [G].

This chord diagram represents the proportion of studies dealing with each included sub-branch of the MeSH classification "Diseases" [C] and "Phenomena and Processes" [G], by category. Trials concerning periodontal intervention to improve (or prevent) a systemic condition are classified as A; trials concerning interventions for a better understanding of the links between oral and overall health or observational studies between periodontal disease and a systemic condition are classified as B1+B2. A and B1+B2 correspond to periodontal medicine. Periodontal dentistry (the area of research dealing with periodontal health at mouth level only) is interventional (C) or observational (D).

Trial categories (on the right part of the figure) are shaded either dark (A or C) or light grey (B1+B2 or D). On the left part of the figure, each MeSH sub-branch with its colour code is listed in order of descending frequency. The outer ring of the figure indicates the proportion of studies while the inner circle shows their absolute numbers. In each ring, the contribution of included sub-branches of MeSH [C] and [G] in each trial category is coded by coloured segments. These coloured segments are also sorted by frequency.

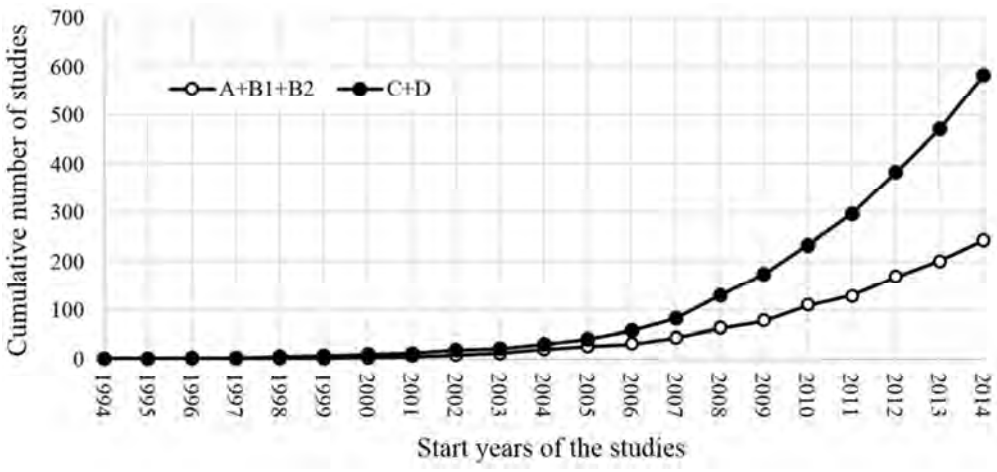
An annotated figure is provided in Supplementary file S2.
164x149mm (300 x 300 DPI)



Phylogenetic-like tree of systemic conditions that have been hypothesized in the registers to be related to periodontal diseases.

Lines from the centre to the periphery of the circle represent the hierarchical tree structure of the MeSH classification. At the end of each taxon, a two-coloured rectangular bar has been added, with length proportional to the relative number of registration records in the A (red) and B1+B2 (grey) categories, respectively.

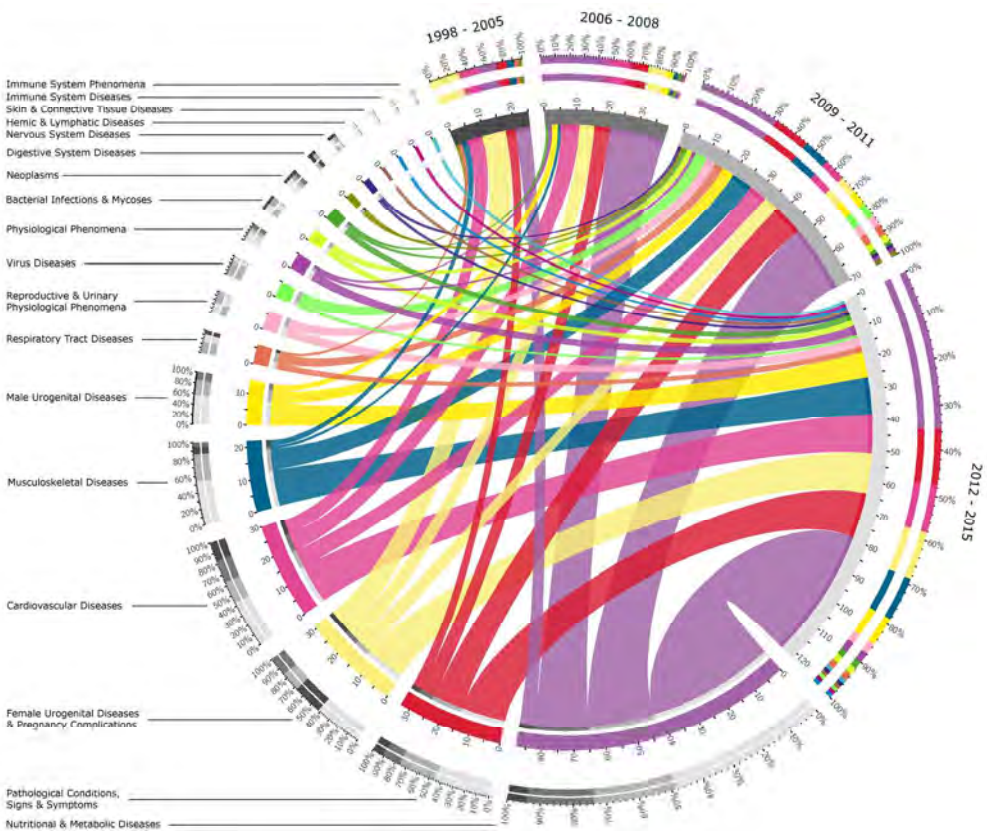
Abbreviations: trials concerning periodontal intervention to improve (or prevent) a systemic condition are classified as A; trials concerning intervention for a better understanding of the links between oral and overall health are classified B1 and observational studies of the link between periodontal disease and a systemic condition are classified B2.
167x123mm (300 x 300 DPI)



Evolution of registration records in periodontal research according to their starting years from the mid-1990's to 2014 and beyond.

On the Y-axis, the cumulative number of studies is represented for each start year noted on the X-axis. Periodontal medicine is represented in orange (A+B1+B2) and periodontal dentistry in blue (C+D). The mean annual growth rate of records between 1998 and 2013 was 39% for periodontal medicine and 35% for periodontal dentistry.

Trials on periodontal intervention to improve (or prevent) a systemic condition are classified as A; trials on intervention for a better understanding of the links between oral and overall health are classified B1 and observational studies of links between periodontal disease and a systemic condition are classified B2. Periodontal intervention to improve oral health is classified as C; observational studies in periodontal research, without systemic assessment are classified as D.
69x32mm (300 x 300 DPI)



Connectogram of the temporal evolution of topics dealing with periodontal medicine.
This chord diagram represents the proportion of studies dealing with each included sub-branch of the MeSH classification "Diseases" [C] and "Phenomena and Processes" [G], linked to the respective start years of the studies (grouped in 4 periods: 1998-2005, 2006-2008, 2009-2011, 2012 and beyond).
164x136mm (300 x 300 DPI)

Table 1

Characteristics of registration records. The number of registered trials for periodontal dentistry and periodontal medicine is detailed by trial register and geographical area, and by median study size.

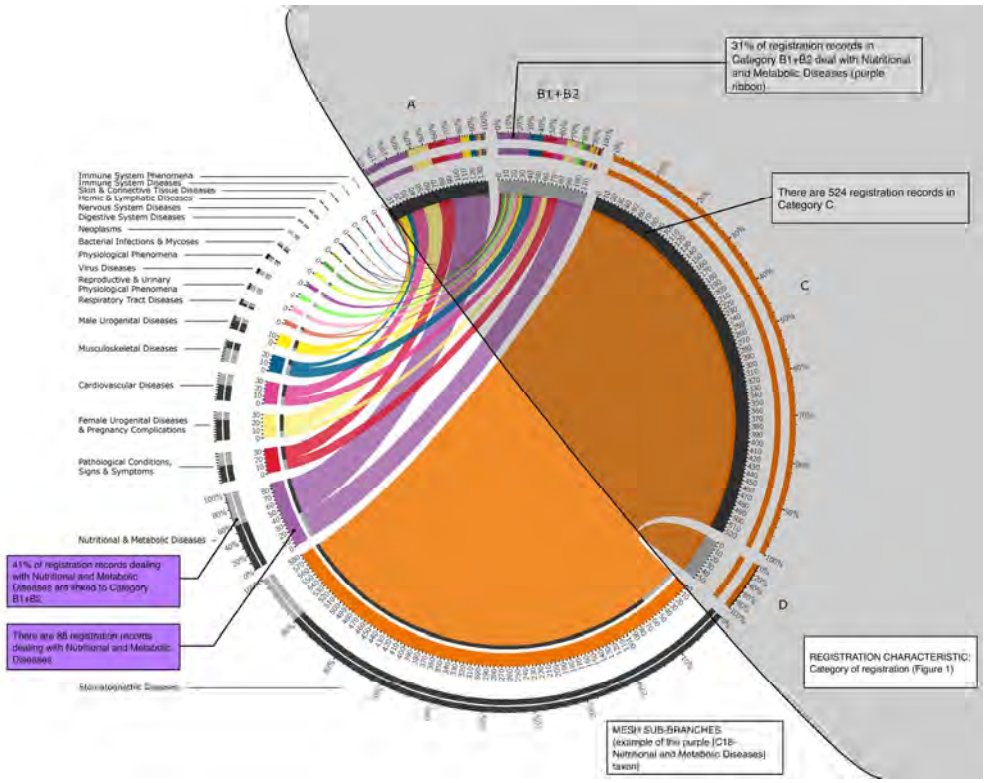
	Periodontal dentistry (N = 580)	Periodontal medicine (N = 242)
Trial register	**580**	**242**
Australian New Zealand Clinical Trials Registry (ANZCTR)	8 (1.4%)	9 (3.7%)
Brazilian Clinical Trials Registry (ReBec)	0 (0%)	5 (2.1%)
Chinese Clinical Trial Register (ChiCTR)	13 (2.3%)	8 (3.3%)
Clinical Research Information Service (CRiS), Republic of Korea	3 (0.5%)	1 (0.4%)
ClinicalTrials.gov	299 (51.6%)	136 (56.2%)
Clinical Trials Registry - India (CTRI)	42 (7.3%)	16 (6.6%)
EU Clinical Trials Register (EU-CTR)	21 (3.6%)	2 (0.8%)
German Clinical Trials Register (DRKS)	10 (1.7%	5 (2.1%)
Iranian Registry of Clinical Trials (IRCT)	98 (16.9%)	21 (8.7%)
ISRCTN.org	24 (4.1%)	17 (7.0%)
Japan Primary Registries Network (JPRN)	39 (6.7%)	19 (7.9%)
Sri Lanka Clinical Trials Registry (SLCTR)	3 (0.5%)	0 (0%)
The Netherlands National Trial Register (NTR)	18 (3.1%)	2 (0.8%)
Thai Clinical Trials Registry (TCTR)	2 (0.3%)	1 (0.4%)
Cuban Public Registry of Clinical Trials	0 (0%)	0 (0%)
Pan African Clinical Trial Registry	0 (0%)	0 (0%)
Areas	**535**	**221**
North America	79 (14.8%)	40 (18.1%)
South America	39 (7.3%)	28 (12.7%)
Europe	128 (23.9%)	46 (20.8%)
Asia	275 (51.4%)	94 (42.5%)
Oceania	6 (1.1%)	12 (5.4%)
Africa	8 (1.5%)	1 (0.5%)
Study size Median [Q1 ; Q3]	48 [30 ; 90]	76 [46 ; 162]

Supplementary File S1

- Bacterial Infections and Mycoses (C01)
  - Bacterial Infections (C01.252)
    - **Bacteremia** (C01.252.100)
    - (~~**Pneumonia. Bacterial**~~) (C01.252.620)
  - Infection (C01.539)
    - (~~**Respiratory Tract Infections**~~) (C01.539.739)
    - (~~Sepsis~~) (C01.539.757)
      - (~~**Bacteremia**~~) (C01.539.757.100)
        - (~~**Endotoxemia**~~) (C01.539.757.100.275)
    - Toxemia (C01.539.861)
      - **Endotoxemia** (C01.539.861.375)
- Virus Diseases (C02)
  - DNA Virus Infections (C02.256)
    - Herpesviridae Infections (C02.256.466)
      - **Epstein-Barr Virus Infections** (C02.256.466.313)
  - RNA Virus Infections (C02.782)
    - Retroviridae Infections (C02.782.815)
      - Lentivirus Infections (C02.782.815.616)
        - **HIV Infections** (C02.782.815.616.400)
  - (~~Sexually Transmitted Diseases~~) (C02.800)
    - (~~Sexually Transmitted Diseases. Viral~~) (C02.800.801)
      - (~~**HIV Infections**~~) (C02.800.801.400)
  - (~~Tumor Virus Infections~~) (C02.928)
    - (~~**Epstein-Barr Virus Infections**~~) (C02.928.313)
- **Neoplasms** (C04)
  - Neoplasms by Site (C04.588)
    - **Breast Neoplasms** (C04.588.180)
    - **Head and Neck Neoplasms** (C04.588.443)
  - (~~Neoplasms. Experimental~~) (C04.619)
    - (~~Tumor Virus Infections~~) (C04.619.935)
      - (~~**Epstein-Barr Virus Infections**~~) (C04.619.935.313)
  - (~~Tumor Virus Infections~~) (C04.925)
    - (~~**Epstein-Barr Virus Infections**~~) (C04.925.313)
- Musculoskeletal Diseases (C05)
  - Bone Diseases (C05.116)
    - **Bone Diseases. Metabolic** (C05.116.198)
      - **Osteoporosis** (C05.116.198.579)
    - (~~Spinal Diseases~~) (C05.116.900)
      - (~~Spondylitis~~) (C05.116.900.853)
        - (~~Spondylarthritis~~) (C05.116.900.853.625)
          - (~~Spondylarthropathies~~) (C05.116.900.853.625.800)
            - (~~**Spondylitis. Ankylosing**~~) (C05.116.900.853.625.800.850)
  - Joint Diseases (C05.550)
    - (~~Ankylosis~~) (C05.550.069)
      - (~~**Spondylitis. Ankylosing**~~) (C05.550.069.680)
    - Arthritis (C05.550.114)
      - **Arthritis. Rheumatoid** (C05.550.114.154)
        - **Sjogren's Syndrome** (C05.550.114.154.774)
      - **Osteoarthritis** (C05.550.114.606)
      - Spondylarthritis (C05.550.114.865)
        - Spondylarthropathies (C05.550.114.865.800)
          - **Spondylitis. Ankylosing** (C05.550.114.865.800.850)
  - (~~Rheumatic Diseases~~) (C05.799)
    - (~~**Arthritis. Rheumatoid**~~) (C05.799.114)
      - (~~**Sjogren's Syndrome**~~) (C05.799.114.774)
    - (~~**Osteoarthritis**~~) (C05.799.613)
- Digestive System Diseases (C06)
  - Gastrointestinal Diseases (C06.405)
    - Esophageal Diseases (C06.405.117)
      - Deglutition Disorders (C06.405.117.119)
        - Esophageal Motility Disorders (C06.405.117.119.500)
          - **Gastroesophageal Reflux** (C06.405.117.119.500.484)
  - Liver Diseases (C06.552)
    - Fatty Liver (C06.552.241)
      - **Non-alcoholic Fatty Liver Disease** (C06.552.241.519)
- (~~Stomatognathic Diseases~~) (C07)
  - (~~Mouth Diseases~~) (C07.465)
    - (~~Salivary Gland Diseases~~) (C07.465.815)
      - (~~Xerostomia~~) (C07.465.815.929)
        - (~~**Sjogren's Syndrome**~~) (C07.465.815.929.669)
- **Respiratory Tract Diseases** (C08)
  - Lung Diseases (C08.381)
    - Lung Diseases. Obstructive (C08.381.495)
      - **Pulmonary Disease. Chronic Obstructive** (C08.381.495.389)
    - (~~Pneumonia~~) (C08.381.677)
      - (~~**Pneumonia. Bacterial**~~) (C08.381.677.540)
  - Respiration Disorders (C08.618)
    - **Respiratory Insufficiency** (C08.618.846)
  - **Respiratory Tract Infections** (C08.730)
    - Pneumonia (C08.730.610)
      - **Pneumonia. Bacterial** (C08.730.610.540)
- Nervous System Diseases (C10)
  - Central Nervous System Diseases (C10.228)
    - Brain Diseases (C10.228.140)
      - **Dementia** (C10.228.140.380)
        - **Alzheimer Disease** (C10.228.140.380.100)
  - (~~Neurodegenerative Diseases~~) (C10.574)
    - (~~Tauopathies~~) (C10.574.945)
      - (~~**Alzheimer Disease**~~) (C10.574.945.249)
- (~~Eye Diseases~~) (C11)
  - (~~Lacrimal Apparatus Diseases~~) (C11.496)
    - (~~Dry Eye Syndromes~~) (C11.496.260)
      - (~~**Sjogren's Syndrome**~~) (C11.496.260.719)
- Male Urogenital Diseases (C12)

- Genital Diseases. Male (C12.294)
  - Infertility (C12.294.365)
    - **Infertility. Male** (C12.294.365.700)
  - Sexual Dysfunction. Physiological (C12.294.644)
    - **Erectile Dysfunction** (C12.294.644.486)
- Urologic Diseases (C12.777)
  - **Kidney Diseases** (C12.777.419)
    - Renal Insufficiency (C12.777.419.780)
      - **Renal Insufficiency. Chronic** (C12.777.419.780.750)
- Female Urogenital Diseases and Pregnancy Complications (C13)
  - Female Urogenital Diseases (C13.351)
    - Urologic Diseases (C13.351.968)
      - **Kidney Diseases** (C13.351.968.419)
        - Renal Insufficiency (C13.351.968.419.780)
          - **Renal Insufficiency. Chronic** (C13.351.968.419.780.750)
  - **Pregnancy Complications** (C13.703)
    - Hypertension. Pregnancy-Induced (C13.703.395)
      - **Pre-Eclampsia** (C13.703.395.249)
    - Obstetric Labor Complications (C13.703.420)
      - Obstetric Labor. Premature (C13.703.420.491)
        - **Premature Birth** (C13.703.420.491.500)
- **Cardiovascular Diseases** (C14)
  - **Heart Diseases** (C14.280)
    - Myocardial Ischemia (C14.280.647)
      - **Coronary Disease** (C14.280.647.250)
        - **Coronary Artery Disease** (C14.280.647.250.260)
  - Vascular Diseases (C14.907)
    - Arterial Occlusive Diseases (C14.907.137)
      - **Arteriosclerosis** (C14.907.137.126)
        - **Atherosclerosis** (C14.907.137.126.307)
          - (~~**Peripheral Arterial Disease**~~) (C14.907.137.126.307.500)
        - (~~**Coronary Artery Disease**~~) (C14.907.137.126.339)
    - **Hypertension** (C14.907.489)
    - (~~Myocardial Ischemia~~) (C14.907.585)
      - (~~**Coronary Disease**~~) (C14.907.585.250)
        - (~~**Coronary Artery Disease**~~) (C14.907.585.250.260)
    - Peripheral Vascular Diseases (C14.907.617)
      - **Peripheral Arterial Disease** (C14.907.617.671)
    - **Prehypertension** (C14.907.653)
- Hemic and Lymphatic Diseases (C15)
  - Hematologic Diseases (C15.378)
    - **Anemia** (C15.378.071)
- Skin and Connective Tissue Diseases (C17)
  - Connective Tissue Diseases (C17.300)
    - **Lupus Erythematosus. Systemic** (C17.300.480)
    - (~~Rheumatic Diseases~~) (C17.300.775)
      - (~~**Arthritis. Rheumatoid**~~) (C17.300.775.099)
        - (~~**Sjogren's Syndrome**~~) (C17.300.775.099.774)
  - (~~Skin Diseases~~) (C17.800)
  - (~~Breast Diseases~~) (C17.800.090)
    - (~~**Breast Neoplasms**~~) (C17.800.090.500)
- Nutritional and Metabolic Diseases (C18)
  - Metabolic Diseases (C18.452)
    - **Glucose Metabolism Disorders** (C18.452.394)
      - **Diabetes Mellitus** (C18.452.394.750)
        - **Diabetes Mellitus. Type 2** (C18.452.394.750.149)
        - **Prediabetic State** (C18.452.394.750.774)
      - (~~Hyperinsulinism~~) (C18.452.394.968)
        - (~~Insulin Resistance~~) (C18.452.394.968.500)
          - (~~**Metabolic Syndrome X**~~) (C18.452.394.968.500.570)
    - Lipid Metabolism Disorders (C18.452.584)
      - Dyslipidemias (C18.452.584.500)
        - **Hyperlipidemias** (C18.452.584.500.500)
    - **Metabolic Syndrome X** (C18.452.625)
  - Nutrition Disorders (C18.654)
    - Malnutrition (C18.654.521)
      - Deficiency Diseases (C18.654.521.500)
        - Avitaminosis (C18.654.521.500.133)
          - **Vitamin D Deficiency** (C18.654.521.500.133.770)
    - Overnutrition (C18.654.726)
      - **Obesity** (C18.654.726.500)
- (~~Endocrine System Diseases~~) (C19)
  - (~~**Diabetes Mellitus**~~) (C19.246)
    - (~~**Diabetes Mellitus. Type 2**~~) (C19.246.300)
    - (~~**Prediabetic State**~~) (C19.246.774)
- **Immune System Diseases** (C20)
  - (~~Autoimmune Diseases~~) (C20.111)
    - (~~**Arthritis. Rheumatoid**~~) (C20.111.199)
      - (~~**Sjogren's Syndrome**~~) (C20.111.199.774)
    - (~~**Lupus Erythematosus. Systemic**~~) (C20.111.590)
  - (~~Immunologic Deficiency Syndromes~~) (C20.673)
    - (~~**HIV Infections**~~) (C20.673.480)
- Pathological Conditions. Signs and Symptoms (C23)
  - Pathologic Processes (C23.550)
    - Disease Attributes (C23.550.291)
      - Disease Susceptibility (C23.550.291.687)
        - **Genetic Predisposition to Disease** (C23.550.291.687.500)
    - **Inflammation** (C23.550.470)
      - (~~Systemic Inflammatory Response Syndrome~~) (C23.550.470.790)
        - (~~Sepsis~~) (C23.550.470.790.500)
          - (~~**Bacteremia**~~) (C23.550.470.790.500.100)
            - (~~**Endotoxemia**~~) (C23.550.470.790.500.100.275)
    - **Yin Deficiency** (C23.550.972)
  - Signs and Symptoms (C23.888)
    - (~~Body Weight~~) (C23.888.144)
      - (~~Overweight~~) (C23.888.144.699)
        - (~~**Obesity**~~) (C23.888.144.699.500)
    - Signs and Symptoms. Digestive (C23.888.821)

- [x] **Dyspepsia** (C23.888.821.236)
- [ ] (~~Diagnosis~~) (E01)
  - [ ] (~~Diagnostic Techniques and Procedures~~) (E01.370)
    - [ ] (~~Physical Examination~~) (E01.370.600)
      - [ ] (~~Body Constitution~~) (E01.370.600.115)
        - [ ] (~~Body Weights and Measures~~) (E01.370.600.115.100)
          - [ ] (~~Body Size~~) (E01.370.600.115.100.160)
            - [ ] (~~Body Weight~~) (E01.370.600.115.100.160.120)
              - [ ] (~~Overweight~~) (E01.370.600.115.100.160.120.699)
                - [ ] (~~**Obesity**~~) (E01.370.600.115.100.160.120.699.500)
- [ ] (~~Mental Disorders~~) (F03)
  - [ ] (~~Delirium, Dementia, Amnestic, Cognitive Disorders~~) (F03.087)
    - [ ] (~~**Dementia**~~) (F03.087.400)
      - [ ] (~~**Alzheimer Disease**~~) (F03.087.400.100)
  - [ ] (~~Sexual and Gender Disorders~~) (F03.800)
    - [ ] (~~Sexual Dysfunctions, Psychological~~) (F03.800.800)
      - [ ] (~~**Erectile Dysfunction**~~) (F03.800.800.400)
- [ ] (~~Genetic Phenomena~~) (G05)
  - [ ] (~~Genotype~~) (G05.380)
    - [ ] (~~**Genetic Predisposition to Disease**~~) (G05.380.355)
- [x] Physiological Phenomena (G07)
  - [ ] (~~Body Constitution~~) (G07.100)
    - [ ] (~~Body Weights and Measures~~) (G07.100.100)
      - [ ] (~~Body Size~~) (G07.100.100.160)
        - [ ] (~~Body Weight~~) (G07.100.100.160.120)
          - [ ] (~~Overweight~~) (G07.100.100.160.120.699)
            - [ ] (~~**Obesity**~~) (G07.100.100.160.120.699.500)
  - [x] Nutritional Physiological Phenomena (G07.610)
    - [x] **Nutritional Status** (G07.610.650)
- [x] Reproductive and Urinary Physiological Phenomena (G08)
  - [x] Reproductive Physiological Phenomena (G08.686)
    - [x] Climacteric (G08.686.157)
      - [x] **Menopause** (G08.686.157.500)
    - [x] Reproductive Physiological Processes (G08.686.785)
      - [x] Reproduction (G08.686.785.760)
        - [x] **Pregnancy** (G08.686.785.760.769)
      - [ ] (~~Sexual Development~~) (G08.686.785.880)
        - [ ] (~~Climacteric~~) (G08.686.785.880.249)
          - [ ] (~~**Menopause**~~) (G08.686.785.880.249.500)
- [x] Immune System Phenomena (G12)
  - [x] Immune System Processes (G12.425)
    - [x] **Transplantation Immunology** (G12.425.901)
- [ ] (~~Population Characteristics~~) (N01)
  - [ ] (~~Demography~~) (N01.224)
    - [ ] (~~Health Status~~) (N01.224.425)
      - [ ] (~~**Nutritional Status**~~) (N01.224.425.525)



186x144mm (300 x 300 DPI)

Supplementary File S3

Exhaustive list of the 966 registration records retrieved from ICTRP with the search strategy, by category (A, B1, B2, C, D, and excluded): registration numbers and titles.

Abbreviations: A - Periodontal intervention to improve (or prevent) a systemic condition; B1 - Intervention for a better understanding of the links between oral and overall health; B2 - Observational study of the possible link between periodontal disease and a systemic condition; C - Periodontal intervention to improve oral health; D - observational studies in periodontal research, without systemic assessment.

Category A: 129 studies
Category B1: 36 studies
Category B2: 77 studies
Category C: 524 studies
Category D: 56 studies
Excluded: 144 studies

Record	Code	Title
ACTRN12605000260628	A	The effect of periodontal treatment and the use of a triclosan dentifrice on glycaemic control in diabetics
ACTRN12605000340639	A	The effect of periodontal treatment and the use of a triclosan containing toothpaste on glycaemic control in diabetes
ACTRN12605000593639	A	The effect of a triclosan containing dentifrice on the relationship between periodontal disease and cardiovascular disease
ACTRN12610000817044	A	Associations between periodontal disease and cardiovascular surrogate endpoints following periodontal treatment in an adult Indigenous population with moderate/severe periodontal disease
ACTRN12612000446864	A	In individuals with both periodontal disease and rheumatoid arthritis does periodontal treatment influence clinical features of rheumatoid arthritis compared with no periodontal treatment?
ACTRN12612001271897	A	In pregnant women how effective is a midwifery intervention (involving oral health education, assessment and referrals to dental clinics) and dental intervention compared with no intervention in improving women's oral health status, uptake of dental services, oral health knowledge, quality of oral health and birth outcomes
ACTRN12614001183673	A	The effect of a periodontal intervention on renal health in Aboriginal Australian adults with kidney disease
ChiCTR-TRC-09000365	A	The effects of periodontal intervention on glucohemoglobin control in patients with periodontits and type 2 diabetics
ChiCTR-TRC-10001062	A	Relationship between periodontal inflammaton and systemic diseases
ChiCTR-TRC-12001913	A	Pre-pregnancy periodontal disease therapy and inflammatory response related to pregnant outcomes
ChiCTR-TRC-13003207	A	Effect of Periodontal Therapy on Lipoproteins Levels in Plasma in Patients with Periodontitis and Hyperlipidaemia
ChiCTR-TRC-13003768	A	Use of Mouth Rinse to Improve Birth and Neonatal Outcomes
ChiCTR-TRC-14004545	A	Research the pathogenesis, prevention and treatment of "Shanghuo"
ChiCTR-TRC-14005045	A	The Effect of Non-Surgical Periodontal Treatment on Systemic Inflammation, Nutritional Status and Lipid level: A Randomized Controlled Trial in End-Stage Renal Disease Patients Undergoing Hemodialysis Therapy
ChiCTR-TRC-14005115	A	The effect of standardized periodontal therapy of the periodontitis on the systemic condition
NCT00012688	A	Periodontal Care and Glycemic Control in Diabetes
NCT00016835	A	Treating Periodontal Infection: Effects on Glycemic Control
NCT00066053	A	Periodontal Intervention for Cardiac Events: A Pilot Trial
NCT00066131	A	Effects of Periodontal Therapy on Preterm Birth
NCT00093236	A	Systemic Endothelial Consequences of Periodontal Disease
NCT00097656	A	MOTOR: Maternal Oral Therapy to Reduce Obstetric Risk
NCT00116974	A	Periodontal Infection and Prematurity Study
NCT00133926	A	Prevention of Pre-Term Birth by Treatment of Periodontal Disease During Pregnancy - The Smile Study
NCT00327561	A	Effects of Non-Surgical Periodontal Therapy on Endothelial Function A Randomized Controlled Clinical Trial
NCT00553007	A	The Relation Between Periodontal Disease and Metabolic Syndrome
NCT00681564	A	Impact of Periodontal Therapy on Endothelial Function
NCT00762762	A	Investigate Plaque and Gingival Index
NCT00763256	A	The Effect of Periodontal Treatment and the Use of Dentifrice on Glycaemic Control in Diabetics
NCT00779909	A	Dose-dependent Anti-inflammatory Effects of Vitamin D in a Human Gingivitis Model
NCT00801164	A	Exploratory Study of Iocide Oral Rinse in a Diabetic Population
NCT00893802	A	Influence of Periodontal Treatment in Pregnant Women Attending Antenatal Care at a Public Health Center in Adverse Pregnant Outcomes: a Controlled Clinical Trial
NCT00937976	A	Impact of Periodontal Therapy on Metabolic and Inflammatory Markers in Chronic Kidney Disease Patients
NCT00997178	A	A Multicenter Randomized Single-Masked Clinical Trial Testing the Effect of Non-surgical Periodontal Therapy on Glycosylated Hemoglobin (HbA1c) Levels in Subjects With Type 2 Diabetes and Chronic Periodontitis
NCT01046435	A	Effects of Periodontal Therapy on Markers of Systemic Inflammation in Subjects at Cardiovascular Disease Risk
NCT01094639	A	Periodontal Infection and Systemic Inflammation in Renal Patients
NCT01128374	A	The Effect of Non-Surgical Periodontal Therapy on Glycemic Control and Bacterial Levels in a Mexican-American Population With Type 2 DiabetesS
NCT01201746	A	Influence of Periodontal Treatment on Systemic Inflammatory Mediators:hsC-reactive Protein, Fibrinogen and White Blood Cells in CHD Patients
NCT01217281	A	The Effect of Non-surgical Periodontal Treatment in the Renal Function of Patients With Chronic Kidney Disease: A Randomized Clinical Trial
NCT01252082	A	Evaluation of Effect of Nonsurgical Periodontal Therapy on Metabolic Control in Patients With Type II Diabetes
NCT01255254	A	The Effect of Oral Hygiene and Full Mouth Scaling on Metabolic Control in Patients With Type II Diabetes
NCT01271231	A	Effects of Periodontal Treatment on Clinical Parameters and Glucose Metabolism in Diabetic Patients
NCT01291875	A	Periodontal Treatment and Metabolic Control in Type 2 Diabetic Patients
NCT01330719	A	Mechanisms and Treatment Response of Aggressive Periodontitis in Children: Aberrant Immunological Phenotypes/Functions in the Progression of AgP
NCT01376791	A	Oral Flora, Periodontitis, and Vascular Dysfunction in Young Native Americans.
NCT01433744	A	Short-term Changes on C-Reactive Protein (CRP) Levels After Non-surgical Periodontal Treatment
NCT01549587	A	A Randomized Controlled Clinical Trial to Evaluate Late First to Mid-Second Trimester Introduction of Advanced Daily Oral Hygiene on Gingivitis and Maternity Outcomes

NCT01568944	A	Peripheral Blood Dendritic Cells and Periodontitis
NCT01609725	A	Impact of Periodontal Therapy in Flow-mediated Dilation and Serum Levels of Cardiovascular Risk Biomarkers in Coronary Artery Disease Patients: 12 Months Randomized Controlled Trial
NCT01639183	A	The Effects of Daily Power Toothbrushing on Caregiver Compliance and on Oral and Systemic Inflammation in a Nursing Home Population: A Randomized Controlled Trial
NCT01641757	A	Effect of Non-Surgical Periodontal Therapy on Serum Albumin Levels of Patients on Maintenance Hemodialysis Therapy, A Randomized Control Trial
NCT01706874	A	Assessment of the Effect of an Intervention (Periodontal Scaling + Mouthwash + Toothpaste) to Reduce the Load on Oral Bacterial Activity of Rheumatoid Arthritis: a Randomized Trial Nested in the Cohort ESPOIR
NCT01785355	A	Periodontal Disease, Inflammation, Nutritional Status And Anaemia Response To Erythropoietin In Chronic Haemodialyzed Patients - The Impact Of Treatment For Periodontal Disease
NCT01798225	A	The Relationships Between Periodontal Disease and Type 2 Diabetes Mellitus in the Gullah Population and the Effects of Mechanical Periodontal Therapy and Systemic Antibiotics on the Glycemic Control
NCT01802216	A	Kidney and Periodontal Disease Study
NCT01881074	A	The Influence of Periodontal Treatment on Gingival Inflammatory Response of the Type II Diabetic Patient
NCT01901926	A	Impact of Non Surgical Periodontal Treatment on Glycemic Control in Type II Diabetics
NCT01904422	A	Evaluation of the Effectiveness of Intensive Periodontal Treatment as Compared to Conventional Periodontal Treatment on the Level of Glycosylated Hemoglobin in Patients With Decompensated Type 2 Diabetes Mellitus: Randomized Clinical Trial
NCT01906450	A	Study on the effects of periodontal treatment along with the use of antibiotics over high sensitive c-reactive protein. Randomized controlled clinical trial.
NCT01917292	A	Periodontal Intervention Improves Vascular Function Among Chinese Prehypertensive Adults With Periodontitis
NCT01951547	A	The Effect of Non-surgical Periodontal Therapy on Host Response and Microbiological Profile of Type 2 Diabetics With Periodontal Disease.
NCT01964833	A	Combination of Photodynamic Therapy and Periodontal Treatment in Patients With Type 2 Diabetes Mellitus: Randomized, Double-blind Clinical Trial
NCT01997814	A	Effect of srp with adjunctive therapy of herbal immunomodulators on the serum c reactive protein (crp) levels & clinical parameters in chronic periodontitis patients - a randomised, double blind, placebo controlled, clinical trial.
NCT01997853	A	Effect of Scaling & Root Planing (SRP) Combined With Therapy Of Omega-3 Fatty Acid on Clinical Parameters and Serum Levels of C-Reactive Protein (CRP) in Chronic Periodontitis - A Randomised Control Trial.
NCT02012842	A	The Effect of Periodontal Treatment on Quality of Life in Patients With Metabolic Syndrome
NCT02014532	A	The Effect Of SRP With Adjunctive Systemic Therapy Of Leukotriene Receptor Antagonist-Montelukast On The Serum C Reactive Protein Levels & Clinical Parameters In Chronic Periodontitis Patients - A Randomized Controlled Trial
NCT02062047	A	Full-mouth and Partial-mouth Scaling and Root Planing in Type 2 Diabetic Subjects: Clinical, Immunological and Microbiological Outcomes
NCT02081976	A	A Randomized, Controlled Trial to Study Effects of Periodontal Therapy in Primary Prevention of Cardiovascular Disease.
NCT02096120	A	Single Arm Pilot Study of Antimicrobial Treatment of Active Rheumatoid Arthritis Associated With Manifest Periodontitis (Translated From German: Anti-mikrobielle Behandlung Der Aktiven Rheumatoiden Arthritis Bei Manifester Parodontitis - Eine Unkontrollierte Therapie-Pilotstudie)
NCT02123563	A	Evaluation of Mechanical-chemical Gingival Therapy in Diabetic, Obese or Diabese Subjects: Quantitative and Qualitative Analysis of Local and General Aspects.
NCT02131922	A	Study of the Effects of Intensive Treatment of Periodontitis on Blood Pressure Control and Vascular Function
NCT02135952	A	Metronidazole and Amoxicillin as Adjuncts to Scaling and Root Planing for the Treatment of Type 2 Diabetic Subjects With Periodontitis: a Randomized Placebo-controlled Clinical Trial
NCT02150005	A	Effects of Non-surgical Periodontal Treatment on Hemogram, Lipid and Glycemic Profiles of Patients With an Indication for Surgical Coronary Revascularization
NCT02172716	A	Disruption of Immune Homeostasis in Type 2 Diabetics With Generalized Chronic Periodontitis
NCT02215473	A	Influence of Pre-procedural Oral Rinse in the Induced Bacteremia by Periodontal Instrumentation: a Randomized Clinical Trial
NCT02220751	A	Diagnostic Biomarkers Related to Periodontal Disease Activity in Diabetic
NCT02263352	A	Systemic Lycopene as an Adjunct to Scaling and Rootplaning in Chronic Periodontitis Patients With Type 2 Diabetes Mellitus
NCT02333227	A	Saving Lives at Birth: Primary Prevention of Periodontal Disease in Relation to Preterm Birth in Malawi (Prevention of Prematurity and Xylitol)
NCT02379975	A	Evaluation of Non-surgical Periodontal Therapy in Patients With Rheumatoid Arthritis
NCT02417376	A	Effect of Nonsurgical Periodontal Treatment on Systemic Risk Markers of Cardiovascular Disease Clinically and Biochemically: A Randomized Trial
CTRI/2011/05/001766	A	Effect of periodontal therapy on the circulating levels of endotoxin in women with periodontitis. A before after clinical trial. - NIL
CTRI/2013/05/003660	A	Effect of non-surgical periodontal therapy on glycaemic control in type II diabetic patients: A randomized controlled clinical trial.
CTRI/2013/10/004051	A	Effects of non-surgical periodontal therapy on insulin resistance in patients with type II diabetes mellitus and chronic periodontitis - a clinical trial
CTRI/2013/11/004136	A	Effect of nonsurgical periodontal therapy in improving the serum iron status of patients with chronic periodontitis - a clinical trial
CTRI/2014/01/004296	A	Effectiveness of periodontal therapy on quality of life, lung function, exacerbation rates and lung inflammation Chronic Obstructive Pulmonary Disorder (COPD) patients- Non randomized control trial
CTRI/2014/08/004849	A	Role of periodontal therapy in glycemic control and its relationship with the inflammatory marker TNF alpha in type 2 diabetic patients-a clinico-biochemical study
CTRI/2014/09/004952	A	Effect of non-surgical periodontal therapy on glycosylated haemoglobin levels in pre diabetic patients with chronic periodontitis
CTRI/2015/02/005581	A	A clinical,biochemical and interventional evaluation of possible relationship between periodontal disease and adverse pregnancy outcomes-A Randomized controlled trial
DRKS00004554	A	Periodontal treatment and reduction of vascular inflammation in patients with peripheral arterial disease
IRCT138706031081N1	A	Effect of periodontal treatment on the C-reactive protein, plasma fibrinogen and white blood cell count in advanced periodontitis -
IRCT138902073811N1	A	Assessment of the outcome of the phase 1 periodontal therapy on prevention of pre-eclampsia -
IRCT138904071081N4	A	Effect of low dose Doxycycline as an adjuct to non-surgical periodontal therapy on the serum level of inflammatory mediators and lipid profile in advanced periodontitis -
IRCT201101291606N2	A	A comparative study on effect of periodontal surgery with rutine periodontal therapy on reduction of serum concentration of CRP
IRCT201103165900N3	A	Evaluation the effect of one stage full mouth disinfection on serum IL17 and IL23 levels in patients with moderate to severe chronic periodontitis -
IRCT201107267128N1	A	Comparison of the effects of one-stage full-mouth disinfection and quadrant-wise scaling and root planing on serum levels of IL-17 and IL-1ß and clinical parameters following treatment of moderate-to-severe chronic periodontitis -
IRCT201202219104N1	A	Effect of phase one of periodontal treatment on improvement of clinical signs of Rheumatoid Arthritis
IRCT201203189316N1	A	Periodontal status in hemodialysis patients (dialysis center of Shiraz Namazi hospital, Faghihi and Sadra) aspects of clinical, microbiological and immunological and determine their correlation with serum C-Reactive protein levels before and after the crime. -
IRCT2012080510501N1	A	The effect of non-surgical periodontal treatment on salivary and serum visfatin in generalized moderate to severe chronic periodontitis -
IRCT2013042913167N1	A	Evaluation of effects of treatment of periodontal disease on recurrence of dyspeptic symptoms emanating from Helicobacter Pylori in patients who underwent systemic Antibiotic therapy -

IRCT2013051313312N1	A	Comparison of the effects of one-stage full-mouth disinfection and quadrant-wise scaling and aoot planing on serum levels of IL-27 and clinical parameters following treatment of moderate-to-severe chronic periodontitis -
IRCT2013092614774N1	A	Effects of local application of tetracycline gel with SRP on HbA1c & lipid profile in type2 diabetic patients -
IRCT2014062418212N1	A	The Comparison of the effects of Botanical mouthwashes of Miswak/Aloe Vera and Cholorhexidine on dental plaque and gingivitis Index of patients having endotracheal tube hospitalized in the Intensive Care Unit.
IRCT2014082417587N7	A	The effect of non-surgical periodontal therapy plus Doxycycline on HbA1c in patients with type 2 diabetes mellitus (DM).
IRCT2015010517587N8	A	The effect of non-surgical periodontal therapy with adjunctive topical doxycycline gel on HbA1c in patients with type 2 diabetes mellitus(DM). -
IRCT2015010517587N9	A	The effect of non-surgical periodontal therapy plus azithromycin gel on HbA1c in patients with type 2 diabetes mellitus (DM). -
ISRCTN00559156	A	Treatment of periodontitis and metabolic control of patients with diabetes mellitus: a controlled clinical trial
ISRCTN03350903	A	Investigation of the effect of treatment of maternal chronic periodontitis on delivery and low birth weight
ISRCTN10227738	A	Influence of Successful Periodontal Intervention on Renal and Vascular Systems in patients with Chronic Kidney Disease-A Pilot Interventional Randomised Controlled Trial
ISRCTN11742127	A	Efficacy of mechanical scaling and root planning and adjunctive chemotherapy (doxycycline hyclate 20 mg) on systemic health improvement in diabetics
ISRCTN15334496	A	Periodontal treatment for improving glycaemic control in diabetic patients: a randomised controlled trial
ISRCTN34471493	A	Control of periodontal inflammation, systemic inflammatory responses and cognitive decline: a comparative study of standard oral care versus periodontal care
ISRCTN36043780	A	The effect of treatment of periodontitis on markers of cardiovascular diseases: a randomized, single blinded, clinical trial
ISRCTN39062047	A	Intensive treatment for periodontal disease: A model of and therapy for inflammatory vascular dysfunction
ISRCTN52833273	A	Outcomes of Periodontal Therapy in Rheumatoid Arthritis (OPERA)
ISRCTN57210949	A	Eliminating periodontal infection in patients with type 2 diabetes: a single centre non-randomised observational diagnosis and treatment study
ISRCTN59866656	A	Impact of treatment of chronic periodontitis on the serum levels of prohepcidin in patients with chronic kidney disease: Interventional controlled clinical assay
ISRCTN74570187	A	A single-center, blinded, placebo-controlled randomized study of the effect of CRX-150 on serum C-Reactive Protein (CRP) and inflammatory cytokines compared to placebo in subjects with severe adult periodontitis
ISRCTN79186420	A	Efficacy of therapeutic management of periodontitis on the clinical manifestations of rheumatoid arthritis: a randomized controlled trial
ISRCTN83229304	A	Treatment of periodontitis and metabolic control in patients with type 2 diabetes mellitus: a single-centre randomised interventional trial
ISRCTN96523406	A	Use of chlorhexidine gluconate 0.2% oral rinse (CHX) to reduce the incidence of nosocomial lower respiratory tract infections in intubated ICU adult patients
JPRN-UMIN000004281	A	Intervention Research of Periodontal treatmenton for the patients with periodontitis and nonalcoholic steatohepatitis (NASH)
JPRN-UMIN000004775	A	The effect of periodontal care on lipid profiles in type 2 diabetic patients.
JPRN-UMIN000006356	A	Study on the effects of periodontal treatment on metabolic control of type 2 diabetes
JPRN-UMIN000006693	A	Study of glycemic control after periodontal treatment by resolving gingival inflammation in type 2 diabetic patients with periodontal disease
JPRN-UMIN000013278	A	Comparison of the effects of oral hygiene instruction and periodontal treatment on patients with Type 2 diabetes mellitus
JPRN-UMIN000014585	A	Effect of Periodontal Treatment on Glucose Metabolism among middle-aged men and women (Randamized Controled Trial)
JPRN-UMIN000017008	A	Study of the periodontal disease prevalence and risk in SLE patients, and infulence of periodontal treatment
RBR-24t799	A	Effect of nonsurgical periodontal therapy on levels of c-reactive protein in serum of subjects with severe periodontitis
RBR-69yzkb	A	Effect of periodontal treatment on endothelial and microvascular functions and gingival fluid and blood levels of immunoinflammatory biomarkers in type 2 diabetics with severe periodontitis
RBR-8dfrpt	A	Interaction between chronic periodontitis and type II diabetes: A randomized clinical trial to study the impact of periodontal treatment on severity of both pathologies by clinical, metabolic, immunology and microbiology parameters
ACTRN12610000794000	B1	In pregnant women how effective is a midwifery intervention (involving oral health education, assessment and referrals to dental clinics) compared with no midwifery intervention in improving women's oral health status, uptake of dental services, oral health knowledge and quality of oral health.
ACTRN12612000637842	B1	Randomized clinical trial to evaluate the impact of a dental care program in the quality of life of head and neck cancer patients.
NCT00277706	B1	Impact of Parathyroid Hormone (1-34) on Osseous Regeneration in the Oral Cavity
NCT00594334	B1	Effect of Actonel on Periodontal Health of Postmenopausal Women
NCT00731432	B1	The Effect Of A Transmucosal Herbal Periodontal Patch (THPP) on Gingival Inflammation in Diabetic Patients
NCT00763165	B1	Periodontal Disease and Cardiovascular Disease
NCT01154257	B1	Comparison of Foam Swabs Versus Toothbrushes in Removing Dental Plaque From Orally Intubated Mechanically Ventilated Patients
NCT01198509	B1	Role of Oral and Intestinal Microbiota in Rheumatoid Arthritis (RA)
NCT01246596	B1	Chronic Presence of Epstein Barr Virus in Epithelial Cells From Gingiva is Associated With Periodontitis
NCT01405365	B1	The Impact of Obesity on Nonsurgical Periodontal Treatment of Destructive Periodontal Diseases
NCT01422122	B1	The Effect of Vitamin D Supplementation on the Periodontal Health and Associated Outcomes in Pregnant Women
NCT01475435	B1	Oxidative Stress Markers Evaluation Before and After Periodontal Treatment of Diabetics Type 2 Patients With Generalized Chronic Periodontitis and Healthy Periodontium
NCT01533792	B1	Effect of Non-surgical Periodontal Treatment on Pregnant Women With Periodontitis: a Randomized Clinical Trial
NCT01595594	B1	Effect of a PDT Protocol With Multiple Applications as an Adjuvant on the Non Surgical Treatment of Periodontal Disease in Patients With Type 2 Diabetes. A Clinical and Laboratorial Study in Humans
NCT01806974	B1	Multicenter, Prospective Study, on the Consequences of Anti-interleukin 6 Immunotherapy Treatment for Rheumatoid Arthritis on: - Healthy and Pathological Periodontium - The Level of Expression of Some Markers of Inflammation and Periodontal Pathogenic Bacteria in Periodontal Sulci and Periodontal Pockets
NCT01848379	B1	Human Polymorphonuclear Neutrophil (PMN) Cytosolic Signaling and Effector Functions in Patients With Diabetes Mellitus Type 2 and Periodontitis
NCT02174146	B1	Effect of Non-surgical Periodontal Therapy on Leptin and Visfatin Expression in Gingival Tissues of Chronic Periodontitis With and Without Type 2 Diabetes Mellitus: A Study Utilizing ELISA and Real Time PCR
NCT02227485	B1	Evaluation of the Effects of Punica Granatum Plenifora (Golnaar) in Treatment of Gingivitis in Patients With Diabetes Mellitus
NCT02315222	B1	Impact of Dietary Supplements on Oral and Periodontal Wound Healing
NCT02337257	B1	Periodontal and Cardiometabolic Responses to Vitamin D Intervention in African Americans
NCT02357745	B1	Biochemical Evaluation of Plasma,Urine and Salivary Neopterin Levels in Pre and Post-menopausal Women With Periodontitis Following Non-surgical Therapy
NCT02386020	B1	Clinical Efficacy of Locally Delivered Aloe Vera Gel as an Adjunct to Non-surgical Periodontal Therapy in Chronic Periodontitis Subjects With Type 2 Diabetes Mellitus: a Randomized Controlled Clinical Trial
NCT02437747	B1	Local Drug Delivery of Aloe Vera Gel in Chronic Periodontitis Patients With Controlled Diabetes Mellitus
CTRI/2012/05/002694	B1	A Randomized Controlled Trial comparing G32 and Chlorhexidine in treating Pregnancy Gingivitis
EUCTR2004-003755-39-SE	B1	A single center, single-blind, placebo-controlled, randomized study of the effect of CRX-102 compared to placebo on serum C-reactive protein (CRP) and inflammatory cytokines in subjects with severe adult periodontitis - CRX-102
IRCT138903254190N1	B1	Comparison of the immediate Antibacterial Effects of persica , Chlorhexidine and normal saline Mouthwash in ICU patients -

		persica
IRCT138904244365N2	B1	Comparison of the Antibacterial Effects of Matrica and Chlorhexidine Mouthwashes in ICU patients -
IRCT2013032212847N1	B1	Evaluating the effect of sub-antimicrobial dose Doxycycline as an adjuvant on periodontal treatments in diabetics and non diabetics
IRCT2013123116019N1	B1	Examine the effect of Classic Gum and Sensivital Gum toothbrushes on periodontal indexes in pregnant women
ISRCTN19622667	B1	Association of periodontal infection with cardiovaskular risks
JPRN-UMIN000007670	B1	Effect of glycemic control on periodontitis in type 2 diabetic patients with periodontal disease: a pilot study
JPRN-UMIN000008411	B1	Biological effects of oral antimicrobial on systemic bacterial infection caused by full-mouth scaling and root planning: a randomized controlled clinical trial
JPRN-UMIN000013750	B1	Study on effects of cytokine targeted therapy on periodontal condition in patients with rheumatoid arthritis
RBR-4smr5g	B1	Determination of fasting glucose and glycated hemoglobin levels preditives to dental complications occurrence in patients with type 2 diabetes mellitus
RBR-9gfmm5	B1	Evaluation of the effect of two periodontal treatments in the development of periodontal periodontal disease in children with heart disease
TCTR20140602001	B1	Effectiveness of Lifestyle Change plus Dental Care (LCDC) program on improving glycemic and periodontal status in the elderly with type 2 diabetes
NCT00399620	B2	Chromogranin-A-Derived Peptides in the Gingival Crevicular Fluid and the Saliva of Diabetic Patients With or Without Periodontitis
NCT00490165	B2	Associations Between Periodontal and Periapical Inflammation and Pregnancy Outcomes
NCT00531154	B2	Evaluation of the Dental Health Condition in Obesity Patients Before and After Bariatric Surgery
NCT00582374	B2	Periodontal Disease, Inflammatory Markers, and Preterm Birth
NCT00641901	B2	A Study of Oral Health Care Education and Therapy to Reduce Gingivitis During Pregnancy
NCT00702429	B2	Immunoregulation of Periodontal Disease
NCT00750828	B2	Gingival Wetness and Gingival Crevicular Fluid Volume in Patients With Dry Mouth
NCT00855504	B2	Role of Cytokines in Association Between Periodontitis and Preeclampsia During Pregnancy
NCT00982813	B2	Prospective Study of the Influence of Periodontal Diseases on Pre-term Birth: Observation in the Maternity Hospital of Nantes
NCT00990041	B2	Release of Cytokines by Stimulated Peripheral Blood Mononuclear Cells (PBMC) From Chronic Periodontitis Subjects
NCT01045070	B2	Periodontitis and Its Microbiological Agents as Prognostic Factors in Patients With Coronary Heart Disease
NCT01130207	B2	Effects of Bariatric Surgery on the Gastro-intestinal Microflora
NCT01140945	B2	Periodontal Status of Males Attending in Vitro Fertilization Clinic
NCT01154855	B2	Circulatory Microbial Components and Immune Regulators of Patients With Periodontal Disease and Rheumatoid Arthritis
NCT01156155	B2	Periodontal Disease and P. Gingivalis in Rheumatoid Arthritis
NCT01167543	B2	Relationship and Pathophysiology of Gastroesophageal Reflux and Dental/Periodontal Disease
NCT01233765	B2	Analysis of Neutrophil Response in Chronic Periodontitis (Including the Project Entitled 'Pro-Inflammatory Cytokine Production by Neutrophils in Periodontitis Patients')
NCT01246648	B2	Chronic Presence of Epstein Barr Virus in Sulcular and Junctional Epithelial Tissue From Gingiva is Associated With Severe Periodontitis
NCT01358630	B2	Periodontal-, Cardiovascular- and Aneurysmal Diseases.
NCT01399034	B2	Host DNA Methylation Patterns in Association With Periodontal Disease : MiRNA Changes in Obesity
NCT01467674	B2	Malondialdehyde And Antioxidant Levels In Chronic Periodontitis Patients With Type 2 Diabetes Mellitus Under Different Stages Of Metabolic Control
NCT01489839	B2	Biomarkers of Periodontal Disease Progression
NCT01568697	B2	Oral Microbial and Immunological Characterization of Patients With Immune Dysfunction
NCT01584479	B2	Periodontal Disease Prevention Study:A Retrospective Cohort Study to Assess the Effect of Genetics and Dental Preventive Care on Periodontal Disease
NCT01675336	B2	The Association Between Interleukin-1 Gene Variations and Adult Chronic Periodontal Disease in Chinese (Taiwan))
NCT01676545	B2	Genetics of Periodontal Diseases in Chinese
NCT01691638	B2	Genetics of Periodontal Diseases in European Caucasians
NCT01693731	B2	Oral Health in Breast Cancer Survivors on Aromatase Inhibitors
NCT01711385	B2	Screening for Undiagnosed Diabetes in a Dental Setting - A New Paradigm for Dental Practice
NCT01750528	B2	The Prevalence and the Progression of Periodontitis in Ankylosing Spondylitis (AS) Patients Versus Non-AS Population
NCT01812083	B2	Interleukin-1 Receptor Antagonist Gene Polymorphism and Adverse Pregnancy Outcome in Turkish Women
NCT01852240	B2	Periodontal Disease as a Risk Indicator for Erectile Dysfunction - A Cross-sectional Study on 100 Patients
NCT01866761	B2	Analysis of the Relationship Between Periodontal Disease and Atherosclerosis Within a Local Cooperation System of Japanese Medical Services
NCT01878071	B2	The Effect of Obesity on Oral Peri-implant Health - A Cross-Sectional Study
NCT01950962	B2	Role of phospholipid transfer proteins (PLTP) on endotoxemia caused by buccal inflammation
NCT02022865	B2	Correlation Between Inflammatory Markers in Gingival Crevicular Fluid, Serum and Amniotic Fluids in Periodontal Pregnant Women
NCT02109705	B2	Porphyromonas Gingivalis - a Responsible Pathogen in Alzheimer`s Disease?
NCT02127346	B2	Relationship Between Chronic Periodontitis and Vitamin D and Calcium in Men
NCT02177591	B2	Observational Study of the Association Between Periodontal Disease and Cardiovascular Disease
NCT02180932	B2	Towards a Viral Etiology of Periodontal Disease in Relation to Radiotherapy Treatment of Head and Neck Cancers
NCT02184962	B2	Levels of RANKL and OPG in Gingival Crevicular Fluid of Patients With Post-menopausal Osteoporosis / Osteopenia and Periodontal Disease.
NCT02273128	B2	Calcitonin Receptor Gene Polymorphisms in Patients With Osteoporosis and Chronic Periodontitis-An Observational Study
NCT02289066	B2	Impact of Periodontal Disease on Outcomes in Diabetes
NCT02291835	B2	Dietary Intakes and Periodontal Outcomes After Sanative Therapy
NCT02304497	B2	Evaluation of Platelet Rich Fibrin Growth Factors (PRF) in Type 2 Diabetic Patients With Chronic Periodontitis
NCT02316093	B2	The Effects of Obesity on 8-Hydroxy-Deoxyguanosine Levels in Patients With and Without Periodontal Disease
NCT02327533	B2	Gene Expression Profiles in Generalized Aggressive Periodontitis: A Gene Network-based Microarray Analysis
NCT02394860	B2	Influence of Periodontal Diseases on Hemodynamic and Molecular Vascular Parameters in Patients Suffering of Hypertension
NCT02417363	B2	Affiliated Hospital of Stomatology, School of Medicine, Zhejiang University
NCT02423304	B2	A Serological Evaluation of 77A/G and 11A/12A Associated Gene Polymorphisms of MMP-13 in Periodontitis Affected and Healthy Individuals
NCT02433405	B2	Serum Amyloid A Protein And Fetuin A Protein Levels In Periodontal Health And Disease
KCT0001023	B2	Development of molecular diagnostic markers using oral fluid for diagnosis of periodontitis in diabetes : a pilot study
CTRI/2012/06/002748	B2	Prospective Comparative Study to Estimate the Prevalence of Periodontitis in Type 1 and Type 2 Diabetic and Non-Diabetic Patients
CTRI/2013/12/004249	B2	Assessment of periodontal status in renal receipients before and after transplantation -a cohort study
CTRI/2014/01/004356	B2	Does periodontal inflammation affect glycosylated hemoglobin level in otherwise systemically healthy individuals?- a hospital based study
CTRI/2014/08/004898	B2	Evaluation of the effect of periodontitis on c-reactive protein levels in patients with type ii diabetes mellitus.
CTRI/2014/08/004938	B2	comparison of the prevalence and severity of the periodontal diseases between osteoporotic and non osteoporotic subjects A cross

		sectional study
	CTRI/2014/11/005206	B2 Evaluation of salivary L-Plastin in chronic periodontitis associated with type 2 diabetes
1	CTRI/2014/12/005291	B2 Association between periodontits and metabolic syndrome - a hospital based comparative study
2	EUCTR2005-001440-23-SE	B2 A single center, blinded, placebo-controlled, randomized study of the effect of CRx-139 on pocket depth and inflammatory cytokines compared to placebo in subjects with severe adult periodontitis - CRx-139-PE
3	DRKS00000097	B2 Effectiveness of periodontal therapy in HIV-seropositive patients with chronic periodontitis undergoing highly-active antiretroviral therapy - HIV-cP6 study
4	DRKS00000282	B2 Periodontal changes during pregnancy with and without risk for preterm delivery
5	DRKS00005600	B2 Oral Health of patients with stem cell transplantation in childhood
6	DRKS00005746	B2 Clinical cross-sectional study with prospective follow-up to the oral health status before and after lung transplantation
7	IRCT2014110916246N2	B2 Comparison of salivary IL-1 beta in moderate to severe chronic generalized periodontitis among healthy subjects and patients with type 2 diabetes -
8	ISRCTN15683590	B2 Pilot, open-label, observational, prospective study to evaluated the clinical effect of chondroitin sulfate adjunctive therapy in patients with periodontitis
9	JPRN-UMIN000008071	B2 The study on the effects on atherosclerotic diseases by periodontal treatment
10	JPRN-UMIN000008270	B2 A clinical study to discover the involvement of periodontal disease with circulative diseases
11	JPRN-UMIN000008582	B2 Discussion regarding the correlation between periodontal disease and the blood sugar level/arteriosclerosis
12	JPRN-UMIN000010938	B2 Multi-Institutional Collaborative Cohort Study on the improvement of marker for diabetes mellitus and oral malodor by periodontal treatment (MICCS-DOP)
13	JPRN-UMIN000013485	B2 Prospective observational study of the relationship between periodontal disease and lifestyle-related disease monitored by atherosclerosis-related biomarkers
14	JPRN-UMIN000013751	B2 Study on common risk cytokine genes for periodontitis, diabetes mellitus, and rheumatoid arthritis
15	JPRN-UMIN000014877	B2 Analysis of relationship between chronic periodontitis and glucose metabolic parameters (Ehime Dental Diabetes Study)
16	JPRN-UMIN000014886	B2 Prospective cohort study of rheumatoid arthritis and Sjogren's syndrome (Prevention against the development of rheumatoid arthritis, through application of musculoskeletal ultrasonography and treatment for periodontal disease, collaboration with Kanazawa, Chiba and Nagasaki University
17	JPRN-UMIN000017027	B2 A clinical study to discover the involvement of periodontal disease with circulative diseases in Japan and Asian countries
18	NTR1312	B2 Formation of nitrosamines in patients with periodontitis. - Periodontitis and nitrosamine formation
19	NTR2627	B2 The influence of an oral health training programme for early detection, diagnosis and management of oral HIV lesions by primary health care workers in Nairobi East District in Kenya. -
20	ACTRN12607000129482	C A comparison of fluorescence-guided Er: YAG Laser (ERL) and mechanical scaling and root planing (SRP) for non-surgical sub-gingival debridement for the treatment of chronic periodontitis: a controlled prospective clinical study.
21	ACTRN12610000594022	C Clinical efficacy of fish oil as adjunct therapy for patients with chronic periodontitis
22	ACTRN12614000227695	C Use of manuka honey subgingivally, as an adjunct to scaling and root planing (SRP) versus SRP alone, during treatment of chronic periodontitis in adult patients, in order to reduce probing pocket depths and gingival bleeding; a randomised single-blinded split-mouth clinical trial.
23	ACTRN12614001322628	C Effect of commercial mouthwashes versus water and savacol on level of gingival inflammation and plaque accumulation in healthy adults: A parallel randomized placebo controlled single-blind clinical study.
24	ACTRN12615000065594	C Effect of Air Polishing using Erythritol Powder versus conventional ultrasonic and hand scaling on the resolution of residual periodontal pockets and patient comfort during treatment in patients with chronic periodontitis undergoing maintenance therapy
25	ACTRN12615000341527	C Efficacy of the oral probiotic Streptococcus salivarius in managing biofilm formation in patients wearing fixed orthodontic appliances: A double-blind randomized placebo-controlled trial
26	ChiCTR-OCH-14004778	C Clinical comparative study on the efficacy of periodontal endodontic therapy and periodontal treatment alone for severe periodontitis
27	ChiCTR-PCS-12002443	C Biofilm controlling in orthodontic treatment with a visual aided intervention: a RCT
28	ChiCTR-TRC-09000368	C Bone graft combined with fixed splints to preserve periodontitis-affected mobile teeth: A randomized controlled trial
29	ChiCTR-TRC-13003327	C Clinical and microbiological effect of different sequence of debridement-antibiotic usage in treatment of severe periodontitis
30	ChiCTR-TRC-13003576	C In vivo inhibitory effect of Secnidazole on bacteria of periodontitis and pericoronitis
31	ChiCTR-TRC-13003925	C Impact of antimicrobials combined with mechanical debridement on generalized aggressive periodontitis
32	ChiCTR-TRC-13003939	C Efficacy of minimally invasive surgical technique in the treatment of human intrabony defects with or without regenerative materials: a randomized-controlled trial
33	ChiCTR-TRC-13004128	C An evaluation of the assistant efficacy of periodontal treatment for the oral erosion lichen planus and the post-treatment
34	ChiCTR-TRC-14004822	C The application and evaluation of Er, Cr: YSGG laser in the nonsugical treatment of periodontitis
35	ChiCTR-TRC-14004846	C The timing selection of periodontal therapy with Periodontal-Endodontic Combined Lesions teeth
36	ChiCTR-TRC-14005088	C The effect of occlusal adjustment on the periodontium remodeling of anterior teeth with different attachment levels
37	NCT00004640	C Trials to Enhance Elders' Teeth and Oral Health
38	NCT00010634	C Complementary Naturopathic Medicine for Periodontitis
39	NCT00066001	C The Effect of Systemically Administered Metronidazole Alone and in Combination With Professional Supragingival Plaque Removal on Plaque Composition
40	NCT00066027	C Low-Dose Doxycycline Effects on Osteopenic Bone Loss
41	NCT00066066	C Effect of Three Periodontal Therapies in Current Smokers and Non-Smokers
42	NCT00127244	C Outcomes of Traditional and Medical Models of Periodontal Therapy
43	NCT00167466	C Randomized, Double-blind, Placebo-controlled Crossover Trial of the Soladey-3 Toothbrush on Periodontal Disease Indices in Patients With Mild-to-moderate Periodontal Disease
44	NCT00196456	C Photodynamic Lasertherapy (PDT) in Periodontal Treatment (in Vivo)
45	NCT00199290	C A Phase 2, Randomized, Double Blind, Placebo-Controlled, Parallel Group Study to Evaluate the Efficacy and the Safety of Trafermin in Patients With Marginal Periodontitis in Japan
46	NCT00221130	C Clinical Trial of Regenerative Periodontal Tissue by Transplanting Mesenchymal Stem Cells and Osteoblast Cells - I, II Phase-
47	NCT00255970	C Regenerative Therapy for Vertical Defects Comparing Demineralized Freeze Dried Bone Allograft (DFDBA) and Regenafil
48	NCT00296881	C Clinical Outcomes Following Non-Surgical Treatment of Chronic Periodontitis, Using Scaling and Root Planing (SRP) in Conjunction With PerioWave, Compared to SRP Alone
49	NCT00297518	C A Multicenter, Randomized, Examiner Blinded Study of Photoactivated Disinfection With Scaling and Root Planing in the Treatment of Chronic Periodontitis
50	NCT00297531	C Photodynamic Disinfection in Combination With Scaling and Root Planing in the Treatment of Chronic Periodontitis
51	NCT00336661	C A Multicenter, Randomized, Blinded Study of Two Treatments of Photoactivated Disinfection With SRP Against One Treatment of Photoactivated Disinfection With SRP Against SRP Alone in the Treatment of Chronic Periodontitis
52	NCT00371332	C Effect of Adjunctive Treatment With Arestin on the Subgingival Microflora in Patients With Moderate to Advanced Periodontitis
53	NCT00391547	C Phase II Pilot Efficacy Study to Treat Gingivitis
54	NCT00425451	C The Efficacy and Safety of PerioChip Plus (Flurbiprofen/Chlorhexidine) Formulation in the Therapy of Adult Periodontitis
55	NCT00471783	C The Effects of Flossing With a Chlorhexidine Solution on Interproximal Gingivitis: a Randomized Controlled Trial
56	NCT00496847	C Study on the Efficacy and Safety of PERIOGEN in the Treatment of Periodontal Bone Defect - A Double-blind, Controlled, Randomised,Parallel,Multi-centre Study
57	NCT00514657	C Trial in Periodontal Tissue Regeneration Using Fibroblast Growth Factor-2 (Randomised Controlled Phase II Clinical Trial)
58	NCT00529555	C A 9 Month,3-arm Multicenter Clinical Trial of Treatment With Periocline Gel (2.1% Minocycline HCl) for Adjunctive Use to
59		
60		

		Scaling and Root Planing (SRP) in Adults With Periodontal Disease.
	NCT00554034	C The Effect of Herbal Extracts on Inflammatory Enzymes in the Gingiva: a Dose Finding Study.
1	NCT00587834	C A Clinical Trial to Evaluate Gintuit (TM) (Allogeneic Cultured Keratinocytes and Fibroblasts in Bovine Collagen) as an Alternative to Tissue From the Palate to Enhance Oral Soft Tissue Regeneration and Wound Healing
2 3	NCT00632957	C Center for the Biologic Basis of Oral/Systemic Diseases Project 5: Oral Infections: Dietary Regulation of Local and Systemic Inflammatory Responses.
4	NCT00662532	C Multi-Center Phase 3 Trial of Minocycline HCl 1mg Microspheres for the Use in Subjects With Peri-Implantitis
5	NCT00669253	C Randomized Controlled Trial to Evaluate the Efficacy of Er:YAG Laser and Surgical Therapy in Treatment of Chronic Periodontitis
6 7	NCT00670618	C A Prospective, Randomized Clinical Study on the Effects of CPP-ACP Paste on Plaque, Gingivitis and White Spot Lesions in Orthodontic Patients - Part 2
8	NCT00670670	C A Prospective, Randomized Clinical Study on the Effects of CPP-ACP Paste on Plaque, Gingivitis and White Spot Lesions in Orthodontic Patients - Part 1
9 10	NCT00679081	C A Prospective, Randomized, Controlled Pilot Study of CelTx(TM) (Apligraf®) as an Alternative to Tissue From the Palate in the Treatment of Gingival Recession Requiring Root Coverage
11	NCT00681135	C Mechanical Plaque Control and Gingivitis Reduction in Fixed Appliance Patients: Comparison of Different Toothbrushing Protocols
12 13	NCT00689143	C A Phase I, Open-Label, Single Center, Safety/Tolerability and Pharmacokinetic Study of Leukine® Administrated in the Gingiva as Three Single Doses on Separate Days
14	NCT00720707	C Coronally Advanced Flap in Combination With Acellular Dermal Matrix With or Without Enamel Matrix Derivatives for Root Coverage
15	NCT00734708	C Phase 3 Clinical Trial of Periodontal Tissue Regeneration Using Fibroblast Growth Factor-2(Trafermin)
16	NCT00743548	C Comparison of Inter-dental Brush to Dental Floss for Reduction of Plaque and Bleeding in Areas With Intact Papilla: A Clinical Trial
17 18	NCT00748943	C The Clinical Effects of a Mouthwash Containing Chlorine Dioxide on Oral Malodor and Salivary Periodontal and Malodorous Bacteria Using for 7days
19	NCT00757159	C Randomised, Clinical Controlled Study on Treatment of Intra-bony Defects With Enamel Matrix Protein (Emdogain®) vs. Nanocrystalline Hydroxyapatite (Ostim®)
20	NCT00758290	C Clinical Study to Evaluate Dental Plaque
21	NCT00758563	C Train New Examiners Via Modified Gingival Margin Plaque
22	NCT00759031	C Investigation of Dental Plaque and Gingival Index
23	NCT00759187	C Evaluate Clinical Research From Commerical Oral Care Products
24	NCT00761930	C Compare the Clinical Efficacy of Prototype Toothpastes.
25	NCT00762151	C Clinical Research Study to Investigate the Anti-Plaque Effect of a Prototype Toothpaste Containing an Anacor Material Via the MGMPI Method
26	NCT00762515	C Clinical Study to Evaluate the Treatment of Gingivitis of Two Toothpastes
27	NCT00762528	C Compare Anti-inflammatory Dentifrices
28	NCT00762619	C Clinical Study to Examine Brushing on Dental Implants
29	NCT00763048	C Collection of Gingival Crevicular Fluid From Periodontitis Patients
30	NCT00781196	C Effect of Low Dose Oral Folic Acid Supplementation on Phenytoin Induced Gingival Overgrowth: A Randomized Double Blind Controlled Trial.
31	NCT00805558	C Comparison of Moxifloxacin With Ciprofloxacin/Metronidazole as Adjunctive Therapy to Mechanical Treatment of Patients With Chronic Periodontitis
32	NCT00855933	C A Controlled Clinical Study to Determine the Gingivitis Benefit of Flossing
33	NCT00881959	C Multi-center, Post-market, Prospective, Randomized, Examiner-Only-Masked Study of Root Coverage With Acellular Dermal Matrix: Puros® Dermis Versus Alloderm®
34	NCT00885599	C A Randomized Multi-treatment/Controlled Double-blinded Study to Evaluate the Efficacy of a Naturally-derived Mouthrinse in the Treatment of Gingival Inflammation.
35 36	NCT00902876	C A Randomized Controlled Clinical Trial to Evaluate Safety and Effectiveness of CAF + Mucograft® Compared to CAF Alone in Patients With Gingival Recessions.
37	NCT00906776	C Randomised, Controlled, Clinical Study to Compare the Effect of a Combination of Enamel Matrix Proteins and Straumann Bone Ceramic With Autogenous Bone in Deep-wide Intrabony Defects
38	NCT00918060	C Clinical Effects of Locally-delivered Gel Containing Camella Sinensis Extracts
39	NCT00926328	C Comparative Efficacy of a Toothpaste That Reduces Plaque and Gingivitis
40	NCT00932347	C Effect of Mouthwash Containing Camellia Sinensis Extracts on Oral Malodor, Plaque and Papillary Bleeding Indices in Gingivitis Patients.
41	NCT00941668	C Evaluate Inflammation Caused by Gingivitis in Adults
42	NCT00952536	C The Effect of Daily Dietary Intake of Dried Whole Food Concentrates of Fruit, Vegetables and Berries (Juice Plus+) in Improving Clinical Outcomes Following Non-surgical Periodontal Therapy: a Pilot Study.
43 44	NCT00955643	C Periodontal Therapy in Severe Cases of Periodontitis: Preliminary Findings of Non-surgical Instrumentation With or Without Hyperbaric Oxygen Therapy (HBOT)
45	NCT00964860	C A Controlled Clinical Study to Determine the Gingivitis Benefit of Flossing
46	NCT00966953	C Development of Clinical Method to Determination Triclosan Retention in Plaque Following Brushing.
47	NCT00972803	C The Effects of Pistacia Mutica on De Novo Dental Plaque Formation, Gingival Inflammation and Oral Microorganisms
48	NCT01015404	C A Study to Assess the Safety of Using Fibroblast Growth Factor-2 With Periodontal Surgery in Japan (Phase 3)
49	NCT01030666	C Effect of Postsurgical Systemic Doxycycline After Regenerative Periodontal Therapy. A Randomized Placebo-controlled Clinical Trial
50	NCT01034501	C Efficacy of Adjunctive Photodynamic Therapy in Non-surgical Treatment of Chronic Periodontitis: a Randomized , Controlled Clinical Trial
51	NCT01040286	C The Efficacy and Safety of Flurbiprofen Chip Versus Chlorhexidine Chip (Periochip®) in Therapy of Adult Chronic Periodontitis
52	NCT01072201	C To Access the Effects of Mucositis in Adults With Dental Implants
53	NCT01079663	C The Efficacy and Safety of Chlorhexidine Gluconate Chip (Periochip®) in Therapy of Symptoms in Patients With Peri-implantitis
54	NCT01079910	C Clinical Efficacy of an Experimental Toothpaste
55	NCT01082822	C Periodontal Ligament Stem Cell Implantation in the Treatment of Periodontitis
56	NCT01098448	C Control of Periodontal Infections
57	NCT01103102	C Phase II Study: Evaluate Dose-Response of Iocide Oral Rinse in a Human Clinical Trial of Gingival Inflammation and Investigate Effects of Biological Markers Indicative of Systemic Disease
58	NCT01113528	C The Effect of Host Response Modulation Therapy (Omega 3 Plus Low-dose Aspirin) as an Adjunctive Treatment of Chronic Periodontitis (Clinical and Biochemical Study)
59	NCT01118143	C Oral Health in Adults: Association With Attitude, Literacy and Psychological Factors
60	NCT01125007	C Biofilm Capacity Removal and Gingival Abrasion After Utilization Medium and Soft Toothbrushes
	NCT01134081	C Expression of Angiogenic Biomarkers During Healing of Intra-Oral Soft Tissue Engineered Grafts
	NCT01142843	C Clinical Preliminary Evidence of the Efficacy of a Mouthwash Containing 5% Propolis for the Control of Plaque and Gingivitis: Phase II Study

NCT01143610	C	A New Regenerative Therapeutic Approach for Root Coverage: a Randomized Clinical Trial
NCT01175720	C	Clinical Comparison Between Two Surgical Techniques With Acellular Dermal Matrix Graft in the Treatment of Gingival Recessions
NCT01195493	C	Clinical and Microbiological Effects of an Essential Oils Solution Used as an Adjunct to Daily Oral Hygiene Practices in Chronic Periodontitis Patients in Supportive Care
NCT01197105	C	Clinical Evaluation of a Mouthwash Based on Schinus Terebinthifolius (Aroeira) Used by Children With Gingivitis
NCT01211223	C	Effects of the Variation in the Time of Systemic Administration of Metronidazole and Amoxicillin Associated to the Non-surgical Therapy of Chronic Periodontitis.
NCT01215201	C	Adjunctive Non-Surgical Therapy of Inflamed Periodontal Pockets Using Diode Lasers During Maintenance Therapy
NCT01229631	C	The Effect of Daily Dietary Intake of Dried Juice Concentrates of Fruit, Vegetables and Berries (Juice Plus+) Upon Periodontal Outcomes in Chronic Periodontitis: A Multicentre Randomised Controlled Trial
NCT01236950	C	Study of an Essential Oil and a Delmopinol Mouthrinse Effect on Dental Plaque Accumulation Index, Gingivitis Index and on Streptococcus Mutans, Lactobacillus, Aerobic and Anaerobic Oral Bacteria Colony Counts.
NCT01236963	C	Comparison of Efficacy on Interproximal Gingivitis and Dental Plaque Accumulation of an Essential Oils Mouthrinse and Dental Floss.
NCT01249846	C	The Efficacy and Safety of 2.5 mg Chlorhexidine Gluconate Chip (PerioChip®) in Frequent Treatment Versus Routine Treatment in Therapy of Adult Chronic Periodontitis
NCT01256996	C	A Randomized Multicenter Study in the Therapy of Periimplantitis: Scaling Versus Low Abrasive Powder
NCT01282229	C	A Multi-Center Single Blind Study of the Laser Assisted New Attachment Procedure Compared to Scaling and Root Planing Alone, Modified Widman Flap Surgery, and Coronal Debridement Alone in the Treatment of Chronic Periodontitis
NCT01307358	C	A Randomized Parallel Study Comparing the Interproximal Plaque and Gingivitis Effects of Three Interdental Cleaning Modalities
NCT01314729	C	Clinical and Radiographic Comparison and Evaluation of Two Types of Lingual Fixed Retainers on the Health of Periodontium
NCT01317446	C	Effect of an Amine Fluoride/Stannous Fluoride Containing Mouthrinse on Gingival Inflammation,Plaque Development, Discoloration and Bacterial Plaque Composition Over Six Months.
NCT01318928	C	The Treatment of Periodontal Diseases. A Randomized, Blinded, Five Years Follow-up, Four-arm, Placebo Controlled Clinical Intervention Trial
NCT01330082	C	Efficacy of Adjunctive Photodynamic Therapy Using Light-emitting Diode in the Treatment of Chronic Periodontitis
NCT01336179	C	The Efficacy of the Miswak Chewing Sticks (Salvadora Persica)on Plaque Removal and Gingival Health: Randomised Clinical Trial
NCT01357785	C	Periodontal Tissue Regeneration Using Autologous Periodontal Ligament Stem Cells: Randomized Controlled Clinical Trial
NCT01388023	C	Phase 2 Clinical Study of a Day Long Effect of SmellX Palatal Patch Containing A Herbal Formula on Malodor
NCT01411618	C	Efficacy of Essential Oil Mouthwash With and Without Alcohol: a 3-Day Plaque Accumulation Model
NCT01427764	C	Dentin Hypersensitivity Following Non-surgical Periodontal Therapy With Hand or Ultrasonic Instruments
NCT01438333	C	Efficacy of INERSAN in Patients With Chronic Periodontitis as Adjunctive to Full Mouth Disinfection: a Randomized, Double Masked, Placebo Controlled Clinical Trial
NCT01440426	C	Connective Tissue Graft vs Mucograft Collagen Matrix for Coverage of Multiple Gingival Recession Defects Randomized Controlled Clinical Trial Assessing Superiority in Health Related Quality of Life and Non-Inferiority in Root Coverage
NCT01499225	C	A Double-blind, Randomized, Parallel, Placebo-active Controlled, Multi-center Phase II Clinical Trial to Investigate the Efficacy and Safety of YH14642 Following 12-week Oral Administration in Patients With Chronic Periodontal Disease
NCT01509898	C	The Use of Water Jet for Initial Treatment FOR Peri-Implant Disease
NCT01517334	C	Multi-Center Phase 3 Trial of Minocycline HCl 1 mg Microspheres for the Use in Subjects With Peri-Implantitis: Clinical and Microbiological Evaluations
NCT01521260	C	Microbiological and Clinical Evaluation of Different Implant Surface Decontaminating Procedures in the Surgical Treatment of Peri-implantitis; a Double Blind Placebo Controlled Randomized Clinical Study
NCT01522131	C	Treatment of Class II Furcation Defects in the Maxillary and Mandibular Molars With Bioresorbable Collagen Membrane and Laser
NCT01530126	C	Platelet Derived Growth Factor Stimulates Bone Fill and Rate of Attachment Level Gain
NCT01532674	C	Clinical, Microbiological and Biochemical Effects of the Antimicrobial Photodynamic Therapy
NCT01535690	C	Photodynamic Therapy Associated With Full-mouth Ultrasonic Debridement in the Treatment of Severe Chronic Periodontitis: a Randomized-controlled Clinical Trial Running Title: Photodynamic Therapy Associated With Periodontal Debridement
NCT01538927	C	Evaluation of Early Wound Healing Following Use of Fibrin Sealant (FS) in Periodontal Surgery. A Controlled Randomized Clinical Trial.
NCT01539564	C	Multi-Center Phase 3 Trial of Minocycline HCl 1mg Microspheres for the Use in Subjects With Peri-Implantitis
NCT01548469	C	Single Blind, Randomized, Active-controlled Comparative Clinical Trial to Evaluate Clinical Efficacy and Safety Following the Application for 4 Weeks of Bio Mineral Toothpaste in Patients With Mild Periodontitis
NCT01559987	C	A Randomized Parallel Method Development Study Comparing Clinical to Subclinical Effects From Oral Cleaning Modalities
NCT01578603	C	Effects of Two Sugar Substituted Chewing Gums Plus Tooth Brushing on Different Caries and Gingivitis Related Variables: a Double Blind, Randomized Controlled Clinical Trial
NCT01583491	C	Evaluation of Platelet Rich Fibrin Efficacy on Reduction of Periodontal Problems
NCT01593540	C	Clinical Efficacy and Patient Acceptance of Metal and Metal-Free Interdental Brushes: A Controlled Prospective Randomized Study
NCT01598155	C	The Effect of Supragingival Biofilm Control, and the Combination of Supra and Subgingival Biofilm Control in Periodontal Health of Patients Participating in a Periodontal Preventive Maintenance Program - A Randomized Clinical Trial.
NCT01600118	C	Effect of Low-energy Extracorporeal Shock Waves on Periodontal and Endodontic Parameters
NCT01601145	C	Effect of Probiotic on the Plaque pH. A Randomized Double Blind Interventional Study
NCT01630837	C	Frequency of Oral Hygiene in the Maintenance of Gingival Health
NCT01636830	C	The Incidence of Gingival Fissures _ a Crossover Single-blinded Randomized
NCT01637948	C	Controlled Clinical Trial of Traditional Chinese Medicine Mouthrinse In Improving Oral Health in Orthodontic Patients
NCT01642641	C	The Efficacy of Different Surgical Modalities in the Treatment of Periodontitis. A Single-Centre Randomised Controlled Trial
NCT01647282	C	Local Minocycline to Reduce Future Inflammation and Bone Loss in Periodontal Maintenance Patients
NCT01670305	C	Photodynamic Therapy and Periodontal Therapy. A Clinical, Microbiological and Immunoenzymatic Analysis.
NCT01700348	C	An Investigation of the Effects of a Oral Hygiene Regimen in Irregular Flossers on Gingivitis and Plaque
NCT01718912	C	The Effect of an Antibiotic-antifungal Rinse on Periodontal Disease
NCT01742559	C	The Anesthetic Effect of Anterior Middle Superior Alveolar Technique (AMSA) for Non-surgical Periodontal Procedures: a Randomized Controlled Clinical Study
NCT01750801	C	Clinical Evidence Efficacy of a Mouthwash Containing Propolis for the Control of Plaque and Gingivitis: Phase III, Randomized, Double-blind Comparison With Mouthwash Chlorhexidine Base.
NCT01751178	C	Clinical Study to Evaluate the Efficacy of Chlorhexidine Mouthwashes
NCT01778699	C	Anticaries Effect of Probiotic Lactobacillus Brevis CD2 (Lb CD2). A Randomized Double Blind Interventional Study.
NCT01782170	C	Phase II Study: Evaluate Efficacy of Iocide Oral Rinse in a Human Clinical Trial of Gingival Inflammation and Investigate Effects on Biological Markers Indicative of Systemic Disease
NCT01793389	C	Platelet Rich Fibrin in the Treatment of Localized Gingival Recessions: Split-mouth Randomized Clinical Trial
NCT01799226	C	Study of the Anti-Inflammatory Effects of Colgate Total® During an Experimental Gingivitis Model

NCT01808703	C	Clinical Trial of PeriZone PerioPatch in Subjects With Chronic Periodontitis
NCT01811316	C	A Clinical Trial to Evaluate Long-term Efficacy and Safety of Lozenges Containing Lactobacilli Reuteri (Prodentis™) on Gingivitis
NCT01814592	C	Comparison of Two Bilaminar Techniques for Root Covering of Miller´s Recessions Class I and II: A Blind, Randomized Controlled Clinical Trial
NCT01837199	C	Clinical and Microbiological Effects of Adjunctive Metronidazole Plus Amoxicillin in the Treatment of Generalized Chronic Periodontitis: Smokers Versus Non-Smokers.
NCT01848496	C	A Double-blind Randomized Clinical Trial of Two Techniques for Gingival Displacement
NCT01852253	C	Clinical and Microbiological Evaluation Implant Surface Decontamination With 2% Chlorhexidine in the Surgical Treatment of Peri-implantitis; a Double Blind Controlled Randomized Clinical Study
NCT01857804	C	Surgical Treatment of Peri-implantitis With and Without Adjunctive Use of Antibiotics : a Controlled and Randomized Clinical Study
NCT01870362	C	Improvement of Periodontal Health and Reduction in Periodontal Plaque Micro-flora Using a Probiotic Lozenge in Patients With Chronic Periodontitis
NCT01877421	C	A Double-Blind, Randomized, Controlled, Dose Escalation Clinical Trial of an Antiplaque Chewing Gum - Phase 1 Safety and Tolerability and Phase 2a Safety, Tolerability, and Proof of Concept in a Gingivitis Population
NCT01898000	C	Triphala - A New Herbal Mouthwash in Gingivitis: A Randomized Controlled Clinical Trial
NCT01900535	C	A Randomized Clinical Trial to Evaluate and Compare the Efficacy of Triphala Mouthwash With 0.2% Chlorhexidine in Hospitalized Patients With Periodontal Diseases
NCT01902095	C	Evaluation of the Clinical Effects of Tooth Powder on Plaque Induced Gingivitis
NCT01921738	C	The Effects of Systemic and Locally Azithromycin Adjunct to Scaling and Root Planning on Clinical and Microbiological Periodontal Indices in Moderate to Severe Chronic Periodontitis
NCT01929135	C	Short-term Effect of 2% Atorvastatin Dentifrice as an Adjunct to Periodontal Therapy: A Randomized Double-blind Clinical Trial.
NCT01938183	C	Full-mouth Periodontal Debridement With or Without Adjunctive Metronidazole Gel in Smoking Patients With Chronic Periodontitis
NCT01941797	C	Experimental Peri-implant Mucositis in Humans
NCT01943877	C	Propolis In The Treatment Of Chronic Periodontitis - A Clinicomicrobiologic Study
NCT01952301	C	A Randomized, Controlled Clinical Trial to Evaluate a Xenogeneic Collagen Matrix as an Alternative to Free Gingival Grafting for Oral Soft Tissue Augmentation
NCT01954849	C	Assessment of Therapeutic Potential of a Novel Dental Probiotic in Pediatric Patients Affected by Gingivitis
NCT01956656	C	Efficacy Of Lotus Leaves In Management Of Plaque Induced Gingivitis: A 4 Day Double Blind Clinico-Microbiological Trial.
NCT01972399	C	Influence of Laser Therapy Upon Surgical Treatment of Peri-implantitis Lesions.
NCT01976806	C	The Effects of Docosahexaenoic Acid on Periodontitis in Adults: A Pilot Randomized Controlled Trial
NCT02013323	C	Clinical and Microbiological Efficacy of Systemic Ayurvedic Immunomodulator Septilin in the Treatment of Chronic Periodontitis-- A Randomized Controlled Clinical Trial.
NCT02016157	C	Moxifloxacin In Situ Gel as an Adjunct in the Treatment of Periodontal Pocket: A Randomized Clinical Trial.
NCT02018120	C	Comparison of Platelet Rich Fibrin and Connective Tissue Graft in Treatment of Multiple Gingival Recessions
NCT02023840	C	The Use of Erythritol Powder and Locally Derived Metronidazole for the Non-surgical Treatment of Periodontitis: a Split-mouth Randomized Controlled Clinical Trial
NCT02023853	C	The Use of Erythritol Powder and Locally Derived Metronidazole for the Non-surgical Treatment of Peri-implant Mucositis and Peri-implantitis.
NCT02030470	C	Evaluation of Photodynamic Treatment FOTOSAN® Efficacy in Periodontology
NCT02033226	C	Evaluation of Clinical, Anti-Inflammatory and Anti-Infective Properties of Amniotic Membranes Used For Guided Tissue Regeneration in Contained Defects
NCT02039648	C	Proanthocyanidin- Enriched Extract From Rumex Acetosa L. as a Prophylactic Agent Against Intraoral Colonization With Porphyromonas Gingivalis
NCT02043340	C	Effect of A Single Session Of Antimicrobial Photodynamic Therapy Using Indocyanine Green In The Treatment Of Chronic Periodontitis
NCT02048761	C	Locally Delivered 1% Metformin Gel in the Treatment of Intrabony Defects in Subjects With Chronic Periodontitis : A Randomized Controlled Clinical Trial
NCT02049008	C	Clinical, Microbiological and Immunological Effects of Antimicrobial Photodynamic Therapy on Non-surgical Treatment of Aggressive Periodontitis: a Double-blind Split-mouth Randomized Controlled Clinical Trial.
NCT02060032	C	Comparative evaluation of clinical efficacy of subgingivally delivered 1.2% atorvastatin and 1.2% simvastatin in treatment of chronic periodontitis: a randomized controlled trial
NCT02065414	C	Four Weeks Clinical Efficacy of an Ethyl Lauroyl Arginate HCL (LAE) Mouth Rinse: Effect on Gingivitis
NCT02066337	C	Effect of Ozone Gel on Alveolar Bone Density and Superoxide Dismutase in Chronic Periodontitis: A Randomized Controlled Clinical Study
NCT02071199	C	Treatment of Human Gingivitis With Topical ACCS: a Two Week Safety Dose-ranging and Proof-of-principle Trial
NCT02080273	C	The Clinical Investigation of Colgate Total Toothpaste as Compared to Parodontax Toothpaste and Colgate Cavity Protection Toothpaste in Controlling Established Plaque and Gingivitis. (A Six Month Study)
NCT02080403	C	Multi-Center Phase 3 Trial of Chlorhexidine Gluconate Chip for the Use in Subjects With Peri-Implantitis.
NCT02091609	C	Evaluation of Plaque Removal Using Different Plaque Control Therapies Around Single Crown Dental Implants and Splinted Implant Crowns in Patients Under 3 Months Maintenance;a Randomized Clinical Trial
NCT02102295	C	Clinical Study of an Ascorbic Acid Derivative Dentifrice in Patients With Gingivitis
NCT02102360	C	Conventional or Minimally Invasive Surgical Technique for the Treatment of Furcation Defects Using Enamel Matrix Derivative and Anorganic Bovine Bone - a Randomized Controlled Clinical Trial.
NCT02118155	C	Connective Tissue Graft Associated or Not With Low Intensity Laser Therapy: A Randomized Clinical Trial
NCT02119520	C	Platelet rich fibrin combined with 1.2% atorvastatin for treatment of intrabony defects in chronic periodontitis
NCT02120872	C	Platelet - Rich Fibrin Combined With 1.2mg Simvastatin for the Treatment of 3 - Wall Intrabony Defects in Chronic Periodontitis: A Randomized Controlled Clinical Trial
NCT02121665	C	Effect of Probiotic Lozenges as an Adjunct to Non Surgical Periodontal Therapy in Chronic Periodontitis Patients: A Randomized Double Blind Placebo Controlled Clinical and Biochemical Study.
NCT02124655	C	Antiplaque Effect of Essential Oils and 0.2% Chlorhexidine on an in Situ Model of Oral Biofilm Growth: a Randomised Clinical Trial.
NCT02125812	C	Adjunctive Systemic Administration of Moxifloxacin in the Treatment of Aggressive Periodontitis: Double-blind Controlled Clinical Trial
NCT02126267	C	Clinical and Microbiological Evaluation of Techniques for Scaling and Root Planing Per Quadrant and One Stage Full Mouth Disinfection Associated With Azithromycin or Chlorhexidine: Randomized Controlled Trial
NCT02129504	C	Comparison of Two Surgical Techniques to Optimize the Treatment of Gingival Recessions Using a Gingival Graft Substitute.
NCT02135471	C	A 12-month Randomised Controlled Prospective Clinical Study for Root Coverage in Smokers With Acellular Dermal Matrix Graft and Enamel Matrix Derivative
NCT02149758	C	Effect of selective cox-2 inhibitor (etoricoxib) along with scaling and root planing (srp) on clinical parameters and salivary level of superoxide dismutase in chronic generalized periodontitis a double-blind, placebo-controlled, double-masked randomized

			controlled trial (RCT).
	NCT02154594	C	"The Efficacy of Acacia Catechu Mouthrinse as Antiplaque and Antigingivitis Agent in Fixed Orthodontic Appliance Patients.
1	NCT02154906	C	Clinical and Radiographic Evaluation of Demineralized Freeze-Dried Bone Allograft Versus Platelet Rich Fibrin for the Treatment of Periodontal Intrabony Defects in Humans
2	NCT02159781	C	Effect of Periodontal Treatment on Salivary Biomarkers.
3	NCT02160418	C	Incentives for Daily Tooth Brushing to Reduce Gingivitis
4	NCT02160613	C	Effect of Periodontal Surgery on Osteoprotegerin Levels in Gingival Crevicular Fluid, Saliva and Gingival Tissues.
5	NCT02168322	C	Perforated Barrier Membranes Maintain Physiologic Gingival Crevicular Fluid Growth Factor Levels During Treatment of Intrabony Defects: An In Vivo Proof-of-Principle Study
6	NCT02168335	C	The Efficacy of OrasaltsTM in the Treatment of Gingivitis
7	NCT02168543	C	Clinical Efficacy of Subgingivally Delivered 1% Alendronate in the Treatment of Smokers With Chronic Periodontitis: a Randomized Placebo Controlled Clinical Trial.
8	NCT02168621	C	Approaches to Pocket/Root Debridement for Periodontal Infection Control - a Study on Effectiveness.
9	NCT02170857	C	A Randomized Controlled Trial to Evaluate the Effect of Hyaluronic Acid on Infra-bony Defects: A Radiographic and Clinical Study.
10			
11	NCT02174757	C	A Comparative Evaluation of the Effect of Probiotic Inersan and Doxycycline on Chronic Periodontitis - A Clinical and Microbiological Study
12	NCT02185209	C	Surgical Treatment of Peri-implantitis With and Without Systemically Adjunctive Antibiotics A Prospective, Double Blind, Randomized, Three Armed, Parallel, Placebo Controlled Clinical Trial
13			
14	NCT02187016	C	A Randomized, Parallel Design Study to Assess the Effects of Three Interproximal Cleaning Modalities Versus a Manual Toothbrush Control on Gingivitis and Plaque Following a Period of Home Use
15	NCT02187185	C	Studies of Salivary Inflammatory Biomarkers During Biofilm Overgrowth: Confirmation of Predictors and Comparative Effects of Sonicare/Elite-Flexcare in Various Stages of Periodontal Disease
16			
17	NCT02190773	C	Effect of Surgical Periodontal Therapy on the RANKL/OPG System : Do we Need Osteo-immune Modulation Targeting RANKL?
18	NCT02193165	C	The Comparative Efficacy of Three Oral Hygiene Multi-component Regimens Encompassing the Use of a Manual Toothbrush, Toothpaste and a Mouthwash in Controlling Established Dental Plaque and Gingivitis.
19	NCT02195765	C	Two-year Randomized Clinical Trial of Enamel Matrix Derivative Treated Infrabony Defects: Radiographic Analysis
20	NCT02197260	C	Antimicrobial Therapy as Adjunct to Periodontal Treatment: Effect of Timing on Clinical, Microbiological and Systemic Response
21			
22	NCT02203812	C	The Effect of Probiotic Lozenge Administration on Gingivitis and on Mild & Moderate Periodontitis: A Randomized Controlled Clinical Trial.
23	NCT02206009	C	PriMatrix Dermal Repair Scaffold Utilization Intraorally
24	NCT02208739	C	Effect of Nonsurgical Periodontal Therapy Verses Oral Hygiene Instructions on Clinical Parameters as Well as Immunological and Microbial Profile of Patients With Chronic Periodontitis
25			
26	NCT02214095	C	Glucosamine Sulphate as an Adjunctive Therapy to Closed Mechanical Debridement Reduced Gingival Cervicular Fluid IL-1ß in Patients With Chronic Periodontitis
27	NCT02215460	C	Effectiveness of Two Non-surgical Periodontal Treatment Protocols: Randomized Controlled Clinical Trial
28	NCT02218515	C	Evaluation of Gingival Crevicular Fluid Transforming Growth Factor-ß1 Level After Treatment of Intrabony Periodontal Defects With Enamel Matrix Derivatives and Autogenous Bone Graft
29	NCT02223702	C	Clinical Evaluation of Systemic Moxifloxacin Compared to Amoxicillin Plus Metronidazole Adjunct to Non-surgical Treatment in Generalized Aggressive Periodontitis: A Randomized Clinical Trial
30			
31	NCT02229669	C	Coronally Advanced Flap With Two Different Techniques for the Treatment of Multiple Gingival Recessions: A Split-mouth RCT
32	NCT02230787	C	Effect of Emdogain on Wound Healing After Gingival Recession Coverage Using Connective Tissue Graft: A Pilot Study
33	NCT02233998	C	21 Day Clinical Efficacy of Essential Oil Containing Mouth Rinses: Effect on Reducing Existing Gingivitis and Plaque
34	NCT02241577	C	Surgical and Non-surgical Treatment of Peri-implantitis: Randomised Controlled Trial of 12-months Follow-up
35	NCT02242500	C	Phase IV Study of Coronally Advanced Flap With or Without Porcine Collagen Matrix for Treatment of Gingival Recession: a Randomized Controlled Clinical Trial
36	NCT02243046	C	The Clinical Investigation of a Zinc Based Toothpaste as Compared to a Triclosan Based Toothpaste and Colgate Fluoride Toothpaste in Reducing Established Plaque and Gingivitis - a Six-month Study.
37	NCT02248103	C	Comparison of Autogenous Periosteal Pedicle Graft and Collagen Membrane in Management of Periodontal Intrabony Defects: A Randomized Controlled Clinical Trial.
38			
39	NCT02259543	C	Conditioning of Root Surfaces With Citric Acid and Tetracycline for Different Application Times Improves the Outcomes of Root Coverage by Subepithelial Connective Tissue Graft: a Randomized Clinical Trial.
40	NCT02267239	C	Methodology of Application and Immediate Effect of the Essential Oils and 0.2% Chlorhexidine on Oral Biofilm: Immersion Versus Mouthwash.
41			
42	NCT02269748	C	Patient Morbidity and Root Coverage Outcome After Subepithelial Connective Tissue Graft Used in Combination With Coronally Advanced Flap and Tunneling Technique: a Comparative Randomized Controlled Clinical Trial.
43	NCT02274090	C	Local Drug Delivery of 1% Metformin Gel in Moderate and Severe Periodontitis Subjects: a Randomized Controlled Clinical Trial
44			
45	NCT02281071	C	Microsurgical Instruments and Magnification May Enhance Treatment Outcomes Of Laterally Moved, Coronally Advanced Flap in Miller Class III Isolated Recession Defects
46	NCT02283515	C	Efficacy of Locally Delivered 1.2% Rosuvastatin Gel in Non Surgical Treatment of Chronic Periodontitis Patients: A Randomised Clinical Control Trial.
47			
48	NCT02283554	C	Platelet Rich Fibrin With 1% Metformin for the Treatment of Intrabony Defects in Chronic Periodontitis : A Randomized Controlled Clinical Trial
49	NCT02283736	C	Efficacy of Oral Probiotic Administration in Clinical, Immunological and Microbiological Parameters of Patients With Chronic Periodontitis Treated With Non Surgical Periodontal Treatment.
50			
51	NCT02313558	C	Efficacy of a Commercial Dentifrice Containing Ilex Rotunda Thunb Extract for Dental Plaque and Gingivitis: A 3-month Clinical Study in Adults in China
52	NCT02313883	C	A Phase I/II Study to Evaluate the Safety, Tolerability and Efficacy of PerioSept® and Scaling and Root Planing in Subjects With Periodontitis
53	NCT02316652	C	Using Chemical Pocket Disinfection as an Adjunct to Non-surgical Maintenance Therapy of Inflamed Periodontal Pockets
54	NCT02320162	C	School-based Oral Health Education Program Using Experiential Learning or Traditional Lecturing in Children and Adolescents: a Clinical Trial
55			
56	NCT02325570	C	The Evaluation of the Clinical Effects of the KLOX BioPhotonic OraLum Gel With a LED Curing Lamp as an Adjunct to the Non-Surgical Treatment of Moderate to Severe Chronic Periodontitis
57	NCT02329353	C	Clinical And Microbiological Analysis Of Orally Administered Lactobacillus Probiotic Lozenges In Chronic Periodontitis Patients Among Smokers And Non-smokers And Its Correlation With Clinical Parameters - A Clinico-Microbiological Study
58	NCT02335866	C	Platelet Rich Fibrin Against Connective Tissue Graft in Treatment of Gingival Recessions
59	NCT02337166	C	Regeneration of Human Intrabony Defects With Recombinant Human Fibroblast Growth Factor 2 in a Hyaluronic Acid Gel Carrier - a Longitudinal, Prospective, Randomized Controlled Clinical Trial
60	NCT02342691	C	A Phase 1 / 2 Clinical Trial to Assess the Safety and Preliminary Efficacy of Lipoxin Analog BLXA4-ME Oral Rinse for the

		Treatment of Gingivitis
	NCT02355977	C	The Response of Periodontal Pathogens to the Respective or Combined Treatment of Scaling and Root Planning and Locally Delivered Minocycline in Patients With Chronic Periodontitis- a Short-term Randomized Clinical Trial
	NCT02359539	C	Marginal Periosteal Pedicle Flap and Platelet Rich Fibrin Barriers for the Treatment of Periodontal Intrabony Defects - Randomized Clinical Trial
	NCT02359721	C	Efficacy of Clarithromycin is an Adjunct to Scaling and Root Planing .A Clinical Microbiological and Immunological Study.
	NCT02360995	C	The Clinical Investigation of Colgate Total Toothpaste as Compared to Crest Pro-Health Toothpaste and Crest Pro-Health Mouthwash, and Crest Cavity Protection Toothpaste and Crest Fluoride Mouthwash in Reducing Plaque and Gingivitis: A Six-week Clinical Study in the US
	NCT02362854	C	A Randomized Clinical Trial of an Adjunct Diode Laser Application in the Treatment of Peri-implantitis.
	NCT02366585	C	The Effect of Locally Delivered Ciclosporin as an Adjunct to Healing After Treatment of Periodontal Pockets
	NCT02366689	C	Clinical Efficacy in Reducing Established Dental Plaque and Gingivitis of a Toothpaste Containing 0.3% Triclosan, 2% Copolymer / Sodium Fluoride and a Manual Toothbrush as Compared to an Oral Hygiene Multi-component Regimen Encompassing the Use of a Manual Toothbrush, a Toothpaste Containing Stannous Fluoride / Sodium Hexametaphosphate and a Mouthwash Containing 0.07% Cetylpyridinium Chloride
	NCT02368678	C	Effect Off Full Mouth Disinfection And Scaling Root Planing Per Quadrant In Halitosis in Patients With Advanced Chronic Periodontitis: Randomized Controlled Clinical Trial
	NCT02372656	C	Comparison Of Efficacy Of Locally Delivered 1.2% Simvastatin And 1% Metformin Gel In Chronic Periodontitis: A Randomized Placebo Controlled Clinical Trial
	NCT02375178	C	Antimicrobial Activity of Two Mouthwashes
	NCT02375750	C	Surgical Treatment of Peri-implantitis Lesions, With or Without the Placement of a Bone Substitute and a Collagen Membrane. A Randomized Clinical Trial
	NCT02380872	C	Cytokine (IL-1ß) and Matrix Metalloproteinase (MMP) Levels in Gingival Crevicular Fluid After Use of Platelet Rich Fibrin or Connective Tissue Graft in the Treatment of Localized Gingival Recessions
	NCT02385734	C	Concentrated Growth Factor Membrane in the Treatment of Adjacent Multiple Gingival Recessions: A Split Mouth Randomized Clinical Study
	NCT02386033	C	Clinical Efficacy of Subgingivally Delivered Atorvastatin in the Treatment of Mandibular Degree II Furcation Defects: A Randomized Controlled Clinical Trial .
	NCT02390479	C	Gingival Crevicular Fluid Levels of Sclerostin, Osteoprotegerin (OPG) and RANKL in Health, Disease and After Treatment
	NCT02390687	C	Evaluation of the Efficacy of Probiotics in Chronic Periodontitis Patients-A Randomized Placebo Controlled Study.
	NCT02391974	C	Filling of Periodontal Pockets With a Commercially Available Injectable Cross-linked Hyaluronic Acid Dental Filler Versus Oral Hygiene Alone, After Scaling and Root Planing and Chlorhexidine Disinfection, for the Treatment of Periodontitis: A Single-blinded, Multi Center, Prospective, Randomized Controlled Trial
	NCT02393053	C	Innovation Technique With Non-pedicled Buccal Fat Pad Graft in the Treatment of Gingival Recessions: a Randomized Controlled Clinical Trial
	NCT02397122	C	The Adjunctive Effect of Platelet Rich Fibrin to Connective Tissue Graft in the Treatment of Buccal Recession Defects. Results of a Randomized Parallel Group Controlled Trial
	NCT02401360	C	A Controlled Study to Assess Clinical Gingivitis and Microbiome Following the Use of Multiple Oral Hygiene Products
	NCT02402296	C	Efficacy of ß-glucan in Treatment of Localized Aggressive Periodontitis: A Short Term Double-blinded, Placebo-controlled, Randomized Clinical Trial
	NCT02403960	C	The Effect of a Streptococcus Probiotic in Periodontal Therapy: a Randomized Controlled Trial
	NCT02406001	C	Reconstructive Surgical Treatment of Peri-implant Intra-osseous Defects - a multicenter randomized prospective clinical study
	NCT02407379	C	Photoablative-photodynamic (PAPD) Diode Laser Therapy Adjunctive to Scaling and Root Planing in Periodontitis
	NCT02409966	C	Impact of Different Protocols for Treatment of Chronic Periodontitis on the Following Patient-centered Variables: Oral Health Related Quality of Life and Experiences of Fear, Anxiety and Pain: a 6 Month Randomized Clinical Trial
	NCT02412358	C	Clinical Comparison of Three Types of Toothbrushes Pulsar, Crossaction and Butler on Gingivitis and Plaque Removal
	NCT02418520	C	The Effect of Miswak Chewing Sticks on the Oral Helicobacter Pylori Infection
	NCT02433899	C	A Clinical Comparison of Microsurgical Versus Conventional Surgical Approaches for the Semilunar Coronally Advanced Flap
	NCT02433912	C	Clinical Evaluation of Single-stage Advanced Versus Rotated Flaps in the Treatment of Gingival Recessions:Longitudinal, Controlled Clinical Trial.
	NCT02438046	C	Platelet Rich Fibrin in the Treatment of Palatal Wounds After Epithelialized Free Gingival Grafts Harvesting. A Randomized Clinical Trial
	KCT0001366	C	A Double-blind, Randomized, Multicenter, Placebo-controlled Exploratory Clinical Trial of the Efficacy and Safety of "Igatan F Capsule"in gingivitis patients or mild to moderate chronic periodontitis patients
	KCT0001457	C	The clinical and microbial effects of essential oil mouth rinse on periodontal disease
	CTRI/2008/091/000020	C	A randomnized double blinded parallel controlled clinical trial to compare the plaque removal efficacy and gingival health of Anchor toothpaste as compared to regular toothpaste (Colgate dental cream among adult population in Dharwad
	CTRI/2008/091/000285	C	Clinical Evaluation of Bioactive Composite (Chitra-HABG) along with Concentrated Autologous Platelets in the Treatment of Periodontal Defects
	CTRI/2009/091/000006	C	A randomized, double blind, placebo controlled study of efficacy of Lactobacillus CD2 lozenges in patients with chronic periodontitis
	CTRI/2009/091/000626	C	A single centre, randomized, double blinded, controlled experimental gingivitis model to compare the efficacy of 2 product formulations against a regular toothpaste.
	CTRI/2010/091/000035	C	A study to evaluate and compare the efficacy of test mouthwash against non users on the gingival condition and plaque levels after 12 weeks of home use.
	CTRI/2010/091/000617	C	Clinical evaluation of oral health parameters amongst adult subjects.
	CTRI/2011/05/001759	C	Fibrin Sealant Versus Sutures In Periodontal Flap Surgery: A Histo- Morphometric Randomized Pilot Clinical Trial - NIL
	CTRI/2011/05/001767	C	Assessment of Interleukin 1 beta levels and clinical effects of curcumin gel in an experimental gingivitis model. A randomized double blind placebo controlled clinical trial - NIL
	CTRI/2011/05/001774	C	Non-inferiority efficacy study of 2 different formulations of 0.12% Chlorhexidine gluconate oral rinse with (Oroclense) and without alcohol (Oroclear) for treatment of gingivitis.
	CTRI/2011/10/002071	C	comparative evaluation of coronally advanced flap with platelet rich fibrin versus sub-epithelial connective tissue graft in the treatment of gingival recession with Quasi experimental design
	CTRI/2011/12/002187	C	Evaluation of effect of probiotic and combination of probiotic with systemic doxycycline on aggressive periodontitis - a clinical and microbiological study
	CTRI/2012/02/002460	C	An Open label Study to Evaluate the Efficacy and Safety of LPOTC001-11 versus Colgate Plax in dental plaques and gingivitis
	CTRI/2012/04/002610	C	Efficacy of autologous platelet rich fibrin (PRF)and Sree Chitra Hydroxy apatite bioactive glass (HABG)in the management of localized intra bony defects -A randomized controlled Trial.
	CTRI/2012/07/002793	C	A clinical evaluation of efficacy of bone graft material (Beta -tricalcium phosphate + hydroxyapatite) alone and in combination with platelet rich fibrin membrane in treatment of periodontal intrabony defects
	CTRI/2013/01/003286	C	Clinical and radiographic assessment of bone regeneration using alendronate sodium gel as an adjunct to surgical therapy for the treatment of osseous defects in chronic periodontitis- a comparative study
	CTRI/2013/01/003291	C	Comparison of effectiveness of two herbal mouthrinses (Green tea and Guava)on plaque and gingival scores among 12-15 years old school children in Belgaum city-A Randomized Controlled Field Trial.

CTRI/2013/04/003602	C	Impact evaluation of a school-based oral health promotion programme: an evidence in public health practice.
CTRI/2013/05/003677	C	Comparative evaluation of commercially available freeze dried powdered Probiotics on gingival status, plaque inhibition and Mutans streptococci count. A randomized, double blind, clinical study
CTRI/2013/06/003758	C	Vitamin D and Chronic Periodontitis- A Randomised Double Blinded Placebo Controlled Parallel Clinical Trial. - VITDCP
CTRI/2013/06/003772	C	Visible versus Infra Red Low Level LASER therapy in Gingivitis- A double blinded Randomised Controlled Trial
CTRI/2013/09/004013	C	A double blind placebo controlled study to assess the efficacy of acacia arabica phytopharmaceutical gel as an adjunct in treatment and quality of life enhancement of periodontitis patients. - ACA
CTRI/2013/10/004120	C	Effectiveness of two different herbal toothpaste formulations in the reduction of plaque and gingival inflammation in patients with established gingivitis- a randomized controlled trial
CTRI/2013/12/004223	C	Assessment of local Interleukin IL-1 and CCL28 levels and clinical effects of curcumin gel in an experimental gingivitis model. A randomized double blind controlled clinical trial. - CURENEXT
CTRI/2014/01/004334	C	The effectiveness of Ozone water as an adjunct to scaling and root planing for treating periodontitis in comparison with chlorhexidine :A clinical and microbiological study
CTRI/2014/02/004405	C	Autologous platelet rich fibrin with guided tissue regeneration membrane in the treatment of intra-bony defects in chronic periodontitis patients- A randomized, double blind, controlled clinical trial
CTRI/2014/05/004636	C	Efficacy of photodynamic therapy in the management of chronic localized periodontitis in terms of clinical attachment level,fluorescence spectrum and periodontopathogenic bacterial counts-A randomized controlled single blinded clinical trial
CTRI/2014/07/004792	C	The effect of green tea as an adjunct to non surgical management of chronic periodontitis: a randomized clinical trial. - NA
CTRI/2014/08/004844	C	Evaluation of antiplaque and antigingivitis effect of aloe vera, chlorine dioxide and chlorhexidine mouth rinses: a randomised controlled trial
CTRI/2014/08/004855	C	Efficacy of propolis impregnated collagen strip adjuvant to scaling and root planing in chronic periodontitis - a split mouth randomized clinical trial
CTRI/2014/08/004912	C	A Multicenter, Open Label, Randomized, Clinical Study to evaluate and Compare the Efficacy and Safety of Gingivial Paste (Periocream) and Brushing Solution manufactured by Bonyf AG as an adjunct to scaling and roots planning (SRP) with SRP alone in subjects suffering from chronic periodontitis.
CTRI/2014/08/004925	C	Evaluation of the efficacy of green tea catechin gel local drug delivery in the management of chronic periodontitis a quasi experimental study
CTRI/2014/09/004956	C	Efficacy of esterified hyaluronic acid (Hyaloss)in grade II furcation defects- A clinical study
CTRI/2014/09/004977	C	Comparison of effectiveness of Morus alba and chlorhexidine gels on moderate periodontitis in 35 to 55 years of age â?? A hospital based Randomized Controlled Trial
CTRI/2014/10/005145	C	Effectiveness of 5% camellia sinensis (green tea) mouthwash versus 0.12% chlorhexidine mouthwash on plaque induced gingivitis a randomized controlled trial
CTRI/2014/11/005165	C	Evaluation of the clinical efficacy of a Herbal Toothpaste in comparison with a Triclosan containing Toothpaste in a population of dental college students A Double-Blind Randomized Controlled Trial - HTTT
CTRI/2014/11/005203	C	A comparative evaluation of the effect of diode laser and chlorhexidine gel in the management of chronic periodontitis as an adjunct to scaling and root planing:a clinico-microbiological study
CTRI/2014/11/005215	C	A comparative evaluation of the effect of Probiotic Inersan and Doxycycline on Chronic Periodontitis -A Clinical and Microbiological Study
CTRI/2014/12/005237	C	Comparative evaluation of bone regeneration with bone graft and guided tissue regeneration, and bone graft with laser de-epithelialization of flap in the treatment of grade ii furcation defects - a clinical and radiographical study.
CTRI/2014/12/005340	C	An evaluation of egg shell derived hydroxyapatite as bone graft material in the healing of bone defects
CTRI/2015/02/005540	C	Comparison of microsurgical and macrosurgical technique for coverage of localized gingival recession using double papilla flap with connective tissue graft a clinical study.
CTRI/2015/02/005593	C	Role of technology in oral health education among high school children in Mangalore City A Randomized control trial
CTRI/2015/03/005636	C	Effect of soft laser and bioactive glass on bone regeneration in the treatment of human periodontal infrabony defects. A clinical and radiographic study
EUCTR2005-000869-20-ES	C	Post-operative care by chlorhexidine mouthwash after periodontal surgery. Randomised, parallel groups; blind study, DC071BB versus placebo, in patients presenting with periodontal surgery with suture.
EUCTR2005-004457-10-BE	C	Clinical and biological activity of Piascledine® 300 in patients with chronic periodontitis
EUCTR2006-001367-36-DE	C	Benefit of adjunctive systemic postsurgical doxycycline in regenerative periodontal surgery - Effect of postsurgical systemic doxycycline after regenerative periodontal therapy. A randomized pla
EUCTR2006-002192-40-HU	C	New treatment of periodontitis by inhibition of lysine decarboxilase enzyme - New therapy of periodontitis
EUCTR2006-005854-61-DE	C	Adjunctive antimicrobial therapy of periodontitis: Long-term effects on disease progression and oral microbiological colonization - Antibiotics and Periodontitis
EUCTR2006-005883-25-HU	C	Pilot Study on Safety and Explorative Efficacy of MD05 in Comparison with Open Flap Debridement in Patients Undergoing Periodontal Surgery to Treat Deep Intrabony Defects - Scil-MD05-C02
EUCTR2007-005660-27-IT	C	Multi-Center Phase 3 Trial of Minocycline HCl 1mg Microspheres for the Use in Subjects with Peri-Implantitis - NIRVANA
EUCTR2008-000990-39-EE	C	Reduction of the gingival inflammation by V0109 DI. Randomised, parallel groups, double blind study, V0109 DI versus placebo, in patients presenting gingivitis.
EUCTR2008-002982-30-BE	C	A Prospective, randomized, clinical study on the effects of CPP-ACP paste on plaque, gingivitis and initial caries lesion development in orthodontic patients - part 2
EUCTR2008-008512-51-DE	C	A double blind, randomized, placebo-controlled, three-arm, phase II study to investigate the safety and efficacy of oral glycyrrhizin (Glycyron® tablets) for the treatment of gingivitis - Glycyron
EUCTR2009-015914-23-GB	C	Clinical efficacy of an experimental toothpaste
EUCTR2012-000260-17-GR	C	A therapeutic equivalence study, comparing two chlorhexidine digluconate formulations, the test formulation Chlorel® Or. T. Sol. 0,2% w / v and the reference formulation Corsodyl® 0.2% w / v Mint Mouthwash. - INTERMED-CHX-SD2X15
EUCTR2012-002236-87-GB	C	Clinical Study to Evaluate the Efficacy of Chlorhexidine Mouthwashes
EUCTR2012-003430-16-DK	C	Effect of local anesthesia in patients with marginal periodontitis undergoing subgingival depuration - PSD Lozenge
EUCTR2013-001866-40-DE	C	The effect of Zinc-D-gluconate on bleeding propensity of the periodontium in patients with chronic parodontitis - a randomised, placebo-controlled, double-blind interventional study - ParoZink01
EUCTR2013-002190-22-HU	C	Therapeutic clinical trial of three types of “Phlogosol concentrate for gargle” products, comparing their efficacy in reducing different inflammations of the oral cavity.
EUCTR2013-002708-14-DE	C	Phase II study to assess bacterial count reduction of three Octenidine mouthwash concentrations in comparison to a placebo in patients with mild gingivitis - OML-Study
EUCTR2013-003548-22-GB	C	Four Week Clinical Efficacy of An Ethyl Lauroyl Arginate HCL (LAE) Mouth Rinse: Effect on Gingivitis
EUCTR2013-003940-21-NL	C	Systemic antibiotic therapy (amoxicillin plus metronidazole) as an adjunct to surgical treatment of peri-implantitis; a single blind randomized controlled study - Systemic amoxicillin plus metronidazole in surgical peri-implantitis treatment
EUCTR2013-004724-11-SE	C	Surgical treatment of peri-implantitis with and without systemically adjunctive antibiotics. A prospective, open, randomized, three armed, parallel, placebo controlled clinical trial
EUCTR2014-002625-35-SE	C	The effect of locally delivered ciclosporin as an adjunct to healing after treatment of periodontal pockets
DRKS00003270	C	Clinical examination of metal free interdental brushes
DRKS00003285	C	Gingival recession coverage with a modified microsurgical tunnel technique - a prospective randomized controlled clinical trial in man evaluating volumetric and esthetic aspects
DRKS00003954	C	Effects of Motivational Interviewing on periodontal therapy

DRKS00005152	C	A randomized controlled trial assessing the effects of the new erythritol air-polishing powder on microbiological and clinical outcomes during supportive periodontal therapy
DRKS00005389	C	Clinical outcomes of adjunctive antimicrobial photodynamic therapy in the treatment of patients with severe chronic periodontitis - a controlled randomized clinical trial
DRKS00006292	C	Clinical research on photodynamic therapy of gingival hypertrophy in orthodontic patients
IRCT138711091606N1	C	Comparison between Oral B cross action and Oral B advantage on supra gingival plaque removal. - Plaque removal
IRCT138801231809N1	C	The Effects of Subgingival Use of 2/5% Acyclovir Gel on Clinical and Microbiological parameters in Chronic periodontitis -
IRCT138804302216N1	C	Histological evaluation of clinical effect of topical application of barberries dental gel on the gingival inflammation -
IRCT138808142670N1	C	Clinical comparison between coronally advanced flap with amniotic membrane or subepithelial connective tissue grafts in treatment of Miller class and gingival recessions. -
IRCT138808191081N3	C	A study to assess the plaque inhibitory action of a herbal-based toothpaste: A double-blind controlled clinical trial -
IRCT138812093451N1	C	The clinical evaluation of Platelet-Rich Plasma effects on Free Gingival Graft's donor site wound healing -
IRCT138812141248N2	C	Clinical comparison of the localized gingival recession coverage in root surfaces restored with GIOMER and intact root surfaces .
IRCT138901192547N1	C	Evaluation of the effect of Frankincense on moderate plaque induces gingivitis -
IRCT138901192547N2	C	Evaluation of the effect of Thymus concentrated honey in wound healing process after periodontal surgery -
IRCT138901263720N1	C	Comparative study of agents with low and neutral ph as root conditioner and coronally positioned flap in the treatment of human gingival recessions -
IRCT138902043785N1	C	The clinical effect of debridement with combination of PVP-I10% and H2O2 3% in the treatment of moderate to severe chronic periodontitis.
IRCT138902073813N1	C	The Comparison of Farmentin and combined Amoxicilin-Metronidazole in the treatment of Moderate Chronic Periodontits -
IRCT201008244621N1	C	Clinical effect of Parodontax dentifrice on the control of gingivitis and dental plaque -
IRCT201011034877N2	C	Comparative evaluation of microbial colony count of sutures with and without use of periodontal pak after modified widman flap surgery -
IRCT201011261150N4	C	Adjunctive Photodynamic Therapy using Light emitting diode in the Treatment of Advanced Chronic Periodontitis -
IRCT201101015519N1	C	evaluation of Semilunar coronally position flap with free gingival graft for root coverage. -
IRCT201101095570N1	C	The Clinical Effect of Local Delivery of Xanthan-based CHLO-SITE gel with Scaling and Root planning in Chronic Periodontitis -
IRCT201102195861N1	C	Clinical and radiographic evaluation of Bio-Gen with Biocollagen and Bio-Gen with connective tissue in treatment of class II Furcation defects -
IRCT201102245900N1	C	An in vitro/in vivo evaluation of the effect of chlorhexidine in combination with sodium perborate on gingivitis, plaque and tooth surface staining -
IRCT201103136045N1	C	Evaluation of antioxidant effect of green tea in patients with chronic periodontitis on bleeding on probing(BOP) and clinical attachment loss(CAL).
IRCT201104131606N6	C	Design and formulation of periodontal dressing and invivo assesment incompare with Coe-pak - Design and formulation of periodontal dressing
IRCT201105123720N2	C	Comparison of systemic antibiotic alone with scaling and root planing (SRP) in treatment of generalized chronic periodntitis
IRCT201105145570N5	C	Clinical comparison of professional tooth brushing in dental microbial plaque removal using four common manual toothbrushes in Iran -
IRCT201108135305N2	C	Culture of human gingival fibroblasts on an autologous scaffold and evaluation of its effect on augmentation of attached gingiva in comparison with the periosteal fenestration technique in patients with inadequate attached gingiva in a pilot clinical trial -
IRCT201111014877N7	C	Evaluation of efficacy of topical subgingival application of gel prepared from plants of Quercus Brantii L and Coriandrum Sativum L compared with placebo gel on periodontal clinical indices in moderate chronic periodontitis patients. - there is no acronym
IRCT201201094877N10	C	The Effects of Systemic and Locally Azithromycin Adjunct to Scaling and Root Planning on Clinical and Microbiological Periodontal Indices in Moderate to Severe Chronic Periodontitis -
IRCT201201094877N8	C	Clinical trial of effect of toothbrush wear on plaque control in dental faculty students. -
IRCT201201168747N1	C	Clinical evaluation of the efficacy of plasma rich in growth factors with connective tissue graft in treatment of gingival recession -
IRCT201201258825N1	C	Comparative study of clinical and microbial effect of probiotics and phase 1 of periodontal treatment on patient with moderate to severe periodontitis in Tehran university in 1390 -
IRCT201202018723N1	C	Comparison of increasing the width and thickness of keratinized gingiva in two methods of connective tissue graft and graft by composition of collagen and rich plasma in patients who are candidate for gingival grafts -
IRCT201202209086N1	C	Clinical improvement of gingival hyperplasia with mouthwashes cholorhexidine vs persica and powered brush in fixed orthodontic cases - persica vs cholorhexidine vs power brush
IRCT201203229321N1	C	Effectiveness of adjunctive Subantimicrobial Dose Doxycycline on phase I of periodontal therapy -
IRCT201204289570N1	C	The effects of use of Diode (980 nm) laser in pocket therapy on the periodontal health after nonsurgical periodontal therapy -
IRCT201204289582N1	C	Comparative evaluation of salivary substantivity and antiplaque efficacy of mouthwashes containing chlorhexidine, cetylpyridinium chloride and Salvarora Persica in gingivitis patients -
IRCT201205058205N2	C	Evaluation of the effect of doxycycline 20 mg as an adjunct to scaling and root planing in mild periodontitis patients -
IRCT201207019121N2	C	A comparative study of autogenous bone graft use with and without low-level laser therapy in the treatment of the two and three wall intrabony periodontal defects -
IRCT2012070210155N1	C	Comparative Evaluation of Clinical and Microbiological Effect of Subgingival Aplication of Chlorhexidine Gel and Photodynamic Therapy as an Adjunct to Full Mouth Scaling and Root Planning in Treatment of Periodontitis -
IRCT2012070710204N1	C	Effect of sage plant extract (salvia officinalis) gel on dental plaque streptococus mutans microorganism and periodontal indexs.
IRCT2012071710304N1	C	Evaluation of sequences of flossing and brushing on periodontal indices. -
IRCT2012092610942N1	C	Comparison of the effect of echinacea and chlorhexidine mouthwashes on oral health of intubated patients in the Intensive Care Unit -
IRCT2012101611133N1	C	The effect of palatal connective tissue graft usage with and without periosteum as a membrane along with Bio-Oss in treatment of vertical alveolar bone defects -
IRCT2012111139002N3	C	Evaluating the effect of periodontal dressing on wound healing and patient satisfaction after periodontal flap surgery -
IRCT2012111277128N3	C	Comparision of the clinical and microbiological effects of photodynamic therapy as an adjunctive treatment with scaling and root planning with scaling and root planning alone in aggressive periodontitis -
IRCT2012112041081N6	C	Evaluation of placebo effect for pain control during scaling and root planning
IRCT2012121611770N1	C	Comparative evaluation of the clinical and immunological effects of photodynamic therapy as an adjunctive to scaling and root planing and scaling and root planing alone, in the treatment of moderate to severe chronic periodontitis. -
IRCT2012121611771N1	C	The effect of adjunctive low-dose doxycycline and licorice therapy on gingival cervicular fluid matrix metalloproteinase-8 levels in chronic periodontitis - -
IRCT2012121174877N15	C	Compare clinical efficacy of Hygen toothbrushes with and without battery in plaque removing from the teeth surface of students
IRCT2012122611888N1	C	Comparative assessment of effect of traditional oral hygiene and halitosis-base education on oral hygiene indices in Yazd freshman high school students -
IRCT2013020710501N2	C	Comparison of Enamel Matrix Derivatives (EMD) to EMD/Decalcified Freezed Dried Bone Allograft (DFDBA) in the Treatment of Human Intrabony Periodontal Defects: A Controlled Clinical Trial With 6 Month Re-entry -
IRCT2013021612487N1	C	Comparison of antibacterial effect, tooth staining and taste disturbance of 0.2% and 0.12% Chlorhexidine mouth rinses in patients with Gingivitis or mild Periodontitis -
IRCT201302241081N7	C	Effect of Green Tea Mouthwash Containing 1 % Tannin on Plaque, Gingivitis and tooth staining -

	IRCT2013022511771N4	C	Comparison of the effect of adjunctive low dose doxycycline with Omega-3 fatty acid and low-dose Aspirin in chronic periodontitis -
1	IRCT2013050613239N1	C	Evaluation of scaling and root planing effects on salivary melatonin level in patients with chronic periodontitis
2	IRCT201305201760N23	C	The Comparison of Acellular Dermal Matrix Allograft (CenoDerm) and a Subepithelial Connective Tissue Graft for the Treatment of Gingival Recession. -
3	IRCT2013052311771N6	C	Comparison of the effect of adjunctive low dose doxycycline with Omega-3 fatty acid and low-dose Aspirin on gingival crevicular fluid levels of matrix metalloproteinase-8 in chronic periodontitis -
4	IRCT2013052813501N1	C	The effect on the width of keratinized gingiva Mucograft prototype in comparison with the free gingival graft -
5	IRCT2013060313576N1	C	The effect of toothpaste with propolis in Prevention of microbial Plaque formation -
6	IRCT2013061113639N1	C	Comparision of a newly developed laser pen as a home care device versus conventional treatment in reduction of gingival inflammation reduction and acceleration of aphthous and herpetic lesion in up to 15 years old population -
7	IRCT201307248898N2	C	Comparison of ANGIPARS and placebo on enhancement of nonsurgical periodontal treatment outcome in moderate to severe periodontitis -
8	IRCT2013080314255N1	C	Comparative evaluation of immediate effect of root instrumentation with curettes and ultrasonic scalers on Clinical Attachment Level -
9	IRCT2013082314440N1	C	Comparison of effectiveness of four-stage scaling with hand instruments and one-stage ultrasonic in patients with periodontitis -
10	IRCT2013083112487N2	C	Clinical and bacteriologic comparison of scaling & root planning by ultrasonic system with or without photodynamic therapy in patients with mild to moderate chronic periodontitis -
11	IRCT201309303813N3	C	The evaluation of the effect of cocoa on total antioxidant capacity and lipid oxidation in saliva on moderate chronic periodontitis.
12	IRCT2013110115233N1	C	Assessing the effectiveness of chlorhexidine rinse before scaling on the amount of alpha-hemolytic streptococcus and staphylococcus aureus. -
13	IRCT2013110215236N1	C	Treatment of periodontitis and chronic periodontitis and the complications -
14	IRCT2013120915726N1	C	The effect of five oral hygiene teaching method in patients with orthodontic appliances (A comparative evaluation) -
15	IRCT2014010816141N1	C	Effect of Ciprofloxacin versus Amoxicillin and Metronidazole as an additional helpful therapy after scaling and root planning in enhancement of periodontal disease clinical parameters of chronic periodontitis -
16	IRCT2014012216309N1	C	The comparison between chlorhexidine solution and toothbrush in prevention of oral lesions in patients hospitalized in intensive care unit -
17	IRCT2014012816407N1	C	Comparing effect of Chlorhexidine and Persica mouthwashes on color of tooth, gingival bleeding and pain after Scaling in patients suffering gingivitis -
18	IRCT2014020816141N2	C	Evaluation of the effect of Amoxicillin and Metronidazole as an adjunct to full mouth scaling and root planning of chronic periodontitis -
19	IRCT201402164877N18	C	A Clinical Trial of the Comparison Between Clinical Parameters of Patients with Moderate Chronic Periodontitis Using Ultrasonic and Er:YAG -
20	IRCT201403061760N33	C	The Comparison of Free gingival unit graft and free gingival graft for the Treatment of Gingival Recession -
21	IRCT201403155570N6	C	The clinical and patological effect of green tea on the periodontal gingivitis -
22	IRCT2014031817061N1	C	Comparision of the effects of Flower Punica Granatum and Rhus Coriaria Mouthwashes with Cholorhexidine Mouthwash on periodontal indices in 16-25 year fixed orthodontic female patients -
23	IRCT2014040517053N2	C	Effects of Aloe Vera Toothpaste on Periodontal Parameters of Patients with Gingivitis -
24	IRCT2014040617143N1	C	clinical and antibacterial effect of photodynamic therapy and diod laser as adjunctive periodontal therapy in chronic periodontitis -
25	IRCT2014040617145N1	C	Effect of photodynamic therapy and diod laser as adjunctive periodontal therapy on inflammatory mediators levels in gingival cervicular fluid and clinical periodontal status -
26	IRCT201404071760N34	C	The comparison of Papilla preservation technique and pouch& tunnel with subepithelial connective tissue graft in dark triangle treatment -
27	IRCT2014041217228N1	C	Comparison of the effect of low dose doxycycline(20mg capsule) and licorice(490mg tablet) as adjunctive therapy in chronic periodontitis - -
28	IRCT201404133795N2	C	Evaluation of Interleukin 18 levels in saliva of patients with generalized Chronic periodontitis before and after Scaling and root planing -
29	IRCT2014050517587N1	C	Evaluation of localized ibuprofen gel compared with placebo for inhibition of bone loss in chronic periodontitis. -
30	IRCT2014050717587N2	C	Efficacy of subgingivally delivered Doxycycline plus ketoprofen gel as an adjunct to non-surgical treatment of chronic Periodontitis -
31	IRCT2014050817587N3	C	Comparison of a topical piroxicom plus metronidazole gel and piroxicom gel in treatment for pockets in chronic periodontal Disease. -
32	IRCT2014050917587N4	C	A comparative evaluation of the effect of sub-gingival irrigation with ibuprofen 2% mouthwash in treatment of periodontal diseases. -
33	IRCT2014051017587N5	C	Evaluate and compare the efficacy of aloe Vera mouthwash with non- alcoholic Chlorhexidine mouthwash on periodontal diseases. -
34	IRCT2014051717587N6	C	A Comparison of mouth rinse containing alcohol free chlorhexidine with a cetylpyridinium chloride in periodontal diseases -
35	IRCT2014051912487N5	C	Clinical Trial of Comparison of Collagen Membrane and Space Maker With Subepithelial Connective Tissue Graft (Modified Method) in Treatment of Gingival Recession -
36	IRCT2014070918413N2	C	The Effect Of Biocurcumax TM Curcumin (BCM-95) In Patients With Moderate Chronic Periodontitis
37	IRCT2014070918425N1	C	Clinical trial of comparison of analgesic effect of Novafen versus Ibuprofen on pain relief after periodontal surgery -
38	IRCT2014071013167N4	C	Evaluation of effect scaling and root planning on the Trichomonas tenax and Entomoeba gingivalis in patients with chronic periodontitis
39	IRCT2014071518487N1	C	Evaluation the effect of Magnolia mouthwash on the levels of S. mutans in dental plaque and on the Plaque Percent Index -
40	IRCT2014081718834N1	C	Evaluation of effect of phenytoin mucoadhesive paste 1% after non-surgical therapy on improvement of periodontal status in moderate to severe chronic periodontitis patients -
41	IRCT201408176267N2	C	Comparing a desensitizing material and a one-step self-etch adhesive on cervical tooth sensitivity after periodontal surgery: A randomized clinical trial -
42	IRCT20141117143333N24	C	Effect of using and avoiding to use vitamin C on defense system of saliva anti oxidant of periodontal patients -
43	IRCT2014112410155N2	C	The effects of antioxidant supplements melatonin alone and in combination with vitamin C on the clinical characteristics of periodontal treatment phase -
44	ISRCTN07764690	C	Clinical efficacy of subgingival debridement with adjunctive Er:YAG laser in chronic periodontitis patients. A randomised clinical trial.
45	ISRCTN11033714	C	Low-dose doxycycline in the treatment of periodontitis in smokers
46	ISRCTN13093912	C	Comparison of periodontal ligament-derived mesenchymal stem cells embedded in hydroxyapatite-collagen scaffolds versus hydroxyapatite-collagen scaffolds alone in the regenerative treatment of 1 and 2 wall-intrabony defects in patients with chronic periodontitis in terms of clinical attachment level gains.
47	ISRCTN15791152	C	The effectiveness of evidence-based oral hygiene advice and instruction upon patient oral hygiene and self reported behaviour: a randomised controlled trial.
48	ISRCTN17598447	C	Subgingival plaque lipid-A profile as a bacterially-derived biomarker for chronic periodontitis
49	ISRCTN18247441	C	An evaluation of systemic metronidazole and amoxycillin as an adjunct to routine therapy in the treatment of severe periodontal disease
50	ISRCTN19566213	C	Adjunctive benefits of systemic antibiotics to non-surgical treatment of generalised aggressive (early onset) periodontitis: a pilot
51			
52			
53			
54			
55			
56			
57			
58			
59			
60			

		study investigating treatment outcomes and prevalence and persistence of antibiotic resistance.
ISRCTN26530238	C	Antiplaque and antigingivitis effect of lippia sidoides: A double-blind clinical study in humans
ISRCTN28883189	C	Adjunctive Systemic and Locally Delivered Metronidazole in the Treatment of Periodontitis - A Controlled Clinical-Study
ISRCTN29742423	C	Formulation and evaluation of new biodegradable periodontal chip containing Selvadora Persica in chitosan base for the management of chronic periodontitis: a randomized single-blind split mouth study
ISRCTN31193447	C	The effect of Plasma Rich in Growth Factors (PRGF) on periodontal tissue regeneration
ISRCTN35210084	C	Evaluation of a hydrophobic gel adhering to the gingiva in comparison with a standard water soluble 1% chlorhexidine gel after full mouth scaling and root planing in patients with moderate chronic periodontitis: a randomized clinical trial
ISRCTN38542397	C	Is photographic evidence a valuable aide to oral hygiene advice in 9-16 year olds?
ISRCTN39391017	C	A cluster randomized controlled clinical trial of school-based oral health education strategies for adolescents aged 10-13 years
ISRCTN53698147	C	A randomised and controlled cluster trial of 1500 patients attending dental outreach facilities affiliated with the University of Dundee, investigating whether delivery of an enhanced form of oral hygiene instruction, framed using psychological theory with the inclusion of biomarker information, can influence gingival health
ISRCTN55563468	C	A randomised controlled trial to investigate the effects of enhanced oral health advice opon self-reported patient behaviour, oral cleanliness and gingival health
ISRCTN56465715	C	Improving the Quality of Dentistry (IQuaD): A multicentre randomised controlled trial comparing oral hygiene advice and periodontal instrumentation for the prevention and management of periodontal disease in dentate adults attending dental primary care
ISRCTN56889016	C	A randomised controlled trial to examine the effectiveness of single-visit Scale and Polish treatment, delivered at different intervals, in maintaining or improving periodontal health of adult patients
ISRCTN64254080	C	Adjunctive antimicrobial therapy of Periodontitis: long-term effects on disease progression and oral microbiological colonization
ISRCTN67349927	C	The effects of anti-porphyromonas gingivalis egg yolk antibodies in patients with periodontal disease
ISRCTN67470159	C	A pilot study combining individual-based smoking cessation counseling, pharmacotherapy, and dental hygiene intervention
ISRCTN95933794	C	INTERVAL Dental Recalls Trial (Investigation of NICE Technologies for Enabling Risk-Variable-Adjusted-Length Dental Recalls Trial)
ISRCTN98564858	C	Clinical and molecular mechanisms of periodontal wound healing following surgical treatment of localised aggressive periodontitis
JPRN-JMA-IIA00077	C	Efficacy of Enamel Matrix Derivative/ Freeze-Dried Bone Allograft VS Enamel Matrix Derivative/ Demineralized Freeze-Dried Bone Allograft with or without Forced Eruption Treating Infrabony Defects: Two Randomized Trials
JPRN-UMIN000001117	C	Effect of an oral health promotion program on lifestyle modification: a worksite intervention trial
JPRN-UMIN000002684	C	The short-term effect of daily fluoride rinsing in adults: a randomized controlled trial
JPRN-UMIN000003067	C	Personalized medicine of periodontal pathogen
JPRN-UMIN000003484	C	Effect of pocket irrigation with slightly electrolyzed water
JPRN-UMIN000005027	C	Periodontal regeneration with autologous periodontal ligament cell sheets.
JPRN-UMIN000005309	C	A double-blind, randomized controlled pilot study of olive leaf extracts(OLE) for the prevention of periodontal diseases.
JPRN-UMIN000005844	C	Clinical multi-center study of OTC periodontal ointment-containing applicator with brush (MC2) for reducing risk of relapse of chronic periodontitis during supportive periodontal therapy
JPRN-UMIN000005935	C	Transplantation of oral epithelial sheets cultivated on amniotic membrane for oral mucosal reconstruction.
JPRN-UMIN000006407	C	A multi-center randomized controlled clinical trial to examine the clinical, biochemical and antimicrobial effects of a mouth rinse on patients with mild periodontal disease.
JPRN-UMIN000006594	C	Investigation of efficacy and safety for periodontal regeneration using controlled release of platelet-rich plasma impregnated in biodegradable gelatin hydrogel
JPRN-UMIN000006791	C	Effect of supplement jelly intake basic treatment of periodontal disease: Randomized placebo controlled study. (ESTOP Study)
JPRN-UMIN000007312	C	Clinical evaluation of a novel device for the treatment of periodontitis by applying a radical disinfection technique
JPRN-UMIN000007384	C	A randomized controlled clinical trial to examine the microbiological and clinical effects of oral antimicrobials on patients with periodontitis receiving supportive periodontal therapy
JPRN-UMIN000007484	C	Microbiological effect of subgingival ultrasonic instrumentation irrigated with essential oil antiseptic in shallow and deep pockets: a 7-day randomized controlled trial
JPRN-UMIN000007698	C	An exploratory open trial to examine the safety of transplantation of autologous adipose tissue-derived stem cells for periodontal regeneration.
JPRN-UMIN000008231	C	A Non-Inferiority Study Evaluating Fibroblast Growth Factor-2 (KCB-1D) to Enamel Matrix Derivative (Emdogain(R)Gel) for Periodontal Tissue Regeneration.
JPRN-UMIN000008790	C	Healing effects of Er: YAG laser on peri-implant mucositis and peri-implantitis: a clinical randomized controlled trial.
JPRN-UMIN000009436	C	Phase Clinical Trial of mouth rinsing with MA-T107 in patients with periodontitis
JPRN-UMIN000010444	C	Microbiological effect of a 3-step care (brushing, flossing, gargling) on oral cavity niches in chronic periodontitis patients: : a randomized controlled clinical trial
JPRN-UMIN000010753	C	Application of dental LASER to periodontal tissue regenerative therapy
JPRN-UMIN000011511	C	Clinical evaluation of a novel device for the treatment of periodontitis by applying a photolysis of hydrogen peroxide disinfection technique
JPRN-UMIN000011709	C	Effects of collagen membrane for combination therapies with regenerative materials of enamel matrix derivative and xenogeneic bone graft for periodontal surgery.
JPRN-UMIN000012033	C	Development and Validation of Safety of a New Antimicrobial Agent Therapy to early healing for Individual Periodontal Disease
JPRN-UMIN000013376	C	Biological and clinical effect of antimicrobial photo dynamic therapy and local drug delivery system for chronic periodontitis: a randomized controlled clinical trial
JPRN-UMIN000013850	C	Assessment of the effect of administration of propolis and curry leaf into periodontal pockets on oral microbiota
JPRN-UMIN000016207	C	Clinical study for development of the ultrasonic water flow system
JPRN-UMIN000016599	C	A survey of the care effect with appropriate toothbrush choice for people in a hospital or a nursing home
JPRN-UMIN000016725	C	Study of plaque removal rate using electric toothbrush at proximal surface
JPRN-UMIN000016791	C	Clinical trial for evaluation of efficacy and safety of the therapeutic device(RP-14) based on disinfection technique utilizing photolysis of hydrogen peroxide for moderate-severe chronic periodontitis
NTR1215	C	Evaluation of a dentifrice with natural ingredients in the prevention of plaque and gingivitis - N/A
NTR1233	C	Manual toothbrushes and reversal of experimental gingivitis - N/A
NTR1329	C	The plaque inhibitory effect of a CPC mouthrinse in a 3-day plaque accumulation model-A cross-over study - N/A
NTR1429	C	The effect of 1% chlorhexidine gel compared to 0.12% chlorhexidine gel- toothpaste or 0.2% chlorhexidine mouthwash or regular toothpaste in a 3 day non-brushing model on plaque accumulation. - DAGMAR 2 Daily Application of a Gingival Maintenance Antimicrobial Regimen 2
NTR1855	C	A 6-month placebo-controlled CPC-mouthrinse study. -
NTR2038	C	The effect of different interdental cleaning devices on plaque biofilm and gingival bleeding. - N/A
NTR2053	C	Preventive strategies in order to obtain healthy teeth for life. - HT4L
NTR2457	C	The relationship between the incidence of gingival abrasion and the presence of gingival recession in both manual and power brush users. -
NTR2642	C	The effect of a newly developed zendium dentifrice on gingivitis and plaque. Zendium toothpaste. -
NTR2683	C	Conical vs Cylindrical: A study on efficiency of interdental brushes. - CoCy
NTR274	C	The effect of application of 0.12% chlorhexidine gel-toothpaste compared to 0.12% chlorhexidine mouthwash and regular

		toothpaste in a 3 day non-brushing model on plaque accumulation. - DAGMAR Daily Application of a Gingival Maintenance Antimicrobial Regimen
NTR3693	C	Comparison of ultrasonic devices with regard to comfort in periodontal patients during recall. -
NTR3756	C	Non-surgical treatment of peri-implantitis: A randomized controlled trial, single blind study. -
NTR4158	C	Soft rubber bristles interdental cleaner compared to interdental brushes - Soft Rubber Bristles interdental cleaner Evaluation Trail (SORBET)
NTR4165	C	The effect of maltitol sweetened chewing gum on the oral microbiology -RCT- - Bart Keijser Roquette (BAKER)
NTR4208	C	The effect of a dental plaque fluorescence photograph on oral health behavior - Motivation with a QLF photo
NTR4983	C	The effect of Philips Airfloss Ultra plus Listerine compared to dental floss on gingival bleeding, dental plaque, and gingival abrasion in a healing of experimental gingivitis model, a parallel design - APPLE: Airfloss Ultra plus Listerine Evaluated
SLCTR/2010/002	C	A randomized double- blind, placebo- controlled study on the effects of a herbal toothpaste (Sudantha ®) on gingival, oral hygiene and microbial parameters
SLCTR/2010/012	C	A randomized double -blind placebo-controlled study on therapeutic effects of a herbal toothpaste (sudantha) after 6 months use in patients with chronic gingivitis
SLCTR/2011/007	C	Effects of an eugenol containing toothpaste Clogard ® after three months use in patients with chronic gingivitis
TCTR20140621001	C	Effect of acemannan, a polysaccharide extracted from aloe vera gel, on periodontium regeneration in periodontitis patient, a randomized clinical study
TCTR20141028001	C	The effects of probiotic containing lozenges as an adjunct to initial periodontal therapy: 1-year follow up study
ACTRN12611000044921	D	Intra and inter-examiner reproducibility of probing pocket depth with a manual probe in healthy volunteers.
ACTRN12611000129987	D	Intra-examiner reproducibility of probing pocket depth with four manual probes types in healthy volunteers.
ChiCTR-CCS-13004068	D	NLRP3 expression in gingival tissue of periodontitis patient and its significance
ChiCTR-OCH-13004679	D	The study of relationship between biochemical indexes in gingival crevicular fluid, subgingival microbial profiles and the activity of periodontitis
NCT00001726	D	Spatial Organization of Viridans Streptococci in Oral Biofilms
NCT00155571	D	The Occurrence of Periopathogens in Betel-Nut Chewers and the Effects of Areca-Nut on Periopathogens
NCT00162838	D	Effects of Periodontal Pathogens, Porphyromonas Gingivalis and Tannerella Forsythensis, on Cytokine Production From Human Monocyte-Derived Dendritic Cells.
NCT00172744	D	Effect of Cyclic Tensional Force on Osteogenic Differentiation of Human Periodontal Ligament Stem Cells
NCT00277745	D	Integrated Microfluidic System for Oral Diagnostics
NCT00569075	D	Apoptotic Biomarkers of Periodontal Disease
NCT00668746	D	Long-term Safety Evaluation of Minocycline Resistance After Treatment With Minocycline HCl Microspheres, 1 mg in Subjects With Chronic Periodontitis
NCT00969241	D	The Effect of Periodontal Treatment on the Level of Free Radicals in the Saliva
NCT01180920	D	The Importance of Periostin in Periodontal Health and Disease
NCT01234948	D	Oral Malodour and Periodontal Disease-related Parameters. Clinical and Real-time PCR Findings
NCT01379950	D	The Role of Macrophages in the Inflammatory Resolution Phase in Periodontal Patients
NCT01510808	D	The Affect of Orthodontic Treatment on the Periodontal Status of Patients With Aggressive Periodontitis
NCT01599091	D	Characterizing Gingival and Periodontal Ligament Fibroblasts Reaction to Infection With the Perio- Pathogenic Bacteria Porphyromonas Gingivalis
NCT01622192	D	A Comparison Between the Repeatability of Probing Pocket Depths Achieved With Manual and Automated Periodontal Probes
NCT01658475	D	Assessment of Use of Plasma or Serum IgG Test to Screen for Periodontitis
NCT01712672	D	Bacterial Arrangement in Supragingival Biofilms
NCT01742728	D	Oxidative Stress in Community-dwelling Adults
NCT01860495	D	Gingival Crevicular Fluid Bone Morphogenetic Protein - 2 Release Profile Following the Use of Modified Perforated Membrane Barriers in Localized Intrabony Defects (An in Vivo Study)
NCT01888666	D	Comparison Between Rapid and Slow Palatal Expansion: Evaluation of Periodontal Indices
NCT01945632	D	Effect of Root Planing on Surface Topography.
NCT01993368	D	Analysis of Osteoimmune Interactions Linking Inflammation and Bone Destruction in Aggressive Periodontitis
NCT02010307	D	Polymorphonuclear Cells' Sensitivity to Aggregatibacter Actinomycetemcomitans Bacteria in Patients With Aggressive Periodontitis
NCT02013661	D	Clinical Evaluation of Four Types of Suture in Periodontal Surgery
NCT02014857	D	Evaluation of GCF MMP-1, -8 and Growth Factor Levels in Smokers With Periodontal Health
NCT02069574	D	Identification of Novel Periodontal Disease Biomarkers Using microRNA Expression in Saliva
NCT02091258	D	Tooth Loss in Periodontitis Patients on Long-term Maintenance in Private Practice
NCT02111005	D	Influence of Smoking on Fibroblast Apoptosis in Patients With Chronic and Aggressive Periodontitis
NCT02127203	D	Assessment of Nitro - Oxidative Stress in Periodontal Disease
NCT02178046	D	Non-Invasive Oral Biofilm Characterization
NCT02210143	D	An Explorative Study To Develop A Predictive Model Based On Avascular Exposed Root Surface Area For Root Coverage
NCT02280122	D	Activated Matrix Metalloproteinase 8 in Saliva as Diagnostic Test for Periodontal Disease? A Case-control Study
NCT02282800	D	Immunolocalization of 1,25,Dihydroxyvitamin D3 in Aggressive Periodontitis Patients
NCT02403297	D	Rapid Point-of-Care Salivary Diagnostic for Periodontal Health
KCT0001024	D	Identification of novel periodontal disease biomarkers using miRNA expression in saliva
DRKS00003412	D	Oral health among women - a survey
DRKS00003531	D	Differences in DNA-methylation pattern in inflammatory candidate genes in patients with severe generalized periodontitis"
DRKS00005227	D	Clinical and microbiological investigation of the periodontal health in infants, children and adolescents
DRKS00006177	D	Simulation of periodontal pathomechanisms in vitro using parts of human teeth - PDL-LS-2014
IRCT2013053013520N1	D	Design of selective media for the isolation Fusobacterium species in halitosis persons and the etiology of this condition -
IRCT201312304877N17	D	The effect of the Modified Widman Flap surgery on maximum molar bite force in patients with chronic periodontitis -
IRCT201412033451N2	D	Determining concentration of Azitromycin in GCF after single dose administration in healthy subjects (by HPLC method) - Concentration of Azithromycin in GCF
ISRCTN13030013	D	Interleukin 8 and lipoxin a4 levels in the gingival crevicular fluid of smokers and non-smokers with different periodontal diseases: a cross-sectional study
JPRN-UMIN000003342	D	Utility of monitor of diagnosis and therapeutic effect of periodontitis sites that uses GCF and salivary component
JPRN-UMIN000003343	D	Fundamental study to periodontal treatment guideline construction of the next generation using GCF element analysis
JPRN-UMIN000003352	D	The development of the periodontitis recurrence prevention inspection method using GCF
JPRN-UMIN000008954	D	Research on the actual condition survey on dental implant for periodontitis patients
JPRN-UMIN000009290	D	Grasp of the oral hygiene of the elderly people using halitosis measurement.
JPRN-UMIN000012181	D	A study on the clinical application of a new device utilizing antiboy-DEPIM method for the detection of periodontopathic bacteria
JPRN-UMIN000012973	D	Pilot study about utility of blood IgG antibody titer against periodontal pathogen for prediction of development of peri-implantitis
JPRN-UMIN000013691	D	Study of bacterial and serological markers for progression of chronic periodontitis
JPRN-UMIN000015086	D	Survey of periodontal condition in Japanese citizens
NTR4863	D	An exploratory study on the dynamic (microbial, biochemical and immunological) interactions of the oral ecosystem during induction of mild gingival inflammation -
ACTRN12608000092392	Excluded	To measure the efficacy of beta-TriCalcium Phosphate (TCP) bone graft filler in preventing periodontal defect on the distal aspect

		of the M2 following M3 extraction.
ACTRN12609000125224	Excluded	Use of topical immunosupressant,Tacrolimus, in desquamative gingivitis patients: evaluation of effectiveness according to individual and disease characteristics.
ACTRN12609000566235	Excluded	For children with painful infectious mouth conditions and poor oral intake, does topical 2% viscous lignocaine improve oral intake?
ACTRN12610000422022	Excluded	Reducing disease burden and health inequalities arising from chronic disease among Indigenous children: the effect of an early childhood oral education program and fluoride treatment on oral health in indigenous Maori children
ACTRN12610000500055	Excluded	Influence of a pedicle mucoperiosteal flap design on pain, swelling, trismus, alveolar osteitis (dry socket), infection, gingival recession, periodontal pocketing and attachment loss after lower third molar removal
ACTRN12612000630819	Excluded	Comparison of Odontocide with calcium hydroxide paste for interappointment pain in patients undergoing endodontic treatment
ChiCTR-DCS-08000089	Excluded	Compare the accuracy of measuring for axletooth root canal length by two ways Root ZX endometer and X-ray
NCT00001698	Excluded	Randomized, Double Blind, Placebo-Controlled, Phase IIB Trial of Ketorolac Mouth Rinse Evaluating the Effect of Cyclooxygenase Inhibition on Oropharyngeal Leukoplakia: Collaborative Study of the NCI, NIDCD and the NIDCR
NCT00064766	Excluded	A Prospective Randomized Clinical Trial of Doxycycline 20mg Twice a Day Versus Placebo on the Bleeding and Spotting in Women After Insertion of a Levonorgestrel Implant (Norplant) Protocol #2002-1
NCT00099814	Excluded	Clinical/Numerical Study of the Effects of Periodontal Ligament Stress Level on the Rate Bodily Tooth Movement
NCT00104026	Excluded	Genes Associated With Hereditary and Drug-Induced Gingival Overgrowth
NCT00223327	Excluded	Measurement of Bite Force in Humans
NCT00223379	Excluded	Longitudinal Endodontic Study of Apical Preparation Size
NCT00223470	Excluded	Cytokine Regulation of Periradicular Pain in Humans
NCT00226148	Excluded	Immediate Implant Placement in the Molar Regions
NCT00286533	Excluded	Retrospective Evaluation of Implants Placed in the Regular Dentist's Practice. A Study to Evaluate Implant Success, Prosthetic Complications, Opinion of the Patient, and Quality of Care
NCT00379548	Excluded	A Pilot Study of the Changes in Inflammatory State in Asian Americans Changing From Traditional Asian Diet to Typical American Diets
NCT00502606	Excluded	Clinical Study :The Effect of Antibacterial Nanoparticles, Incorporated in Provisional Resin Based Cement, on S.Mutans in the Margins of Provisional Restorations
NCT00762840	Excluded	Clinical Study to Evaluate the Safety and Efficacy of the Apexum Ablator in Subjects With Periapical Lesions Associated With Root Canal Infection
NCT00946634	Excluded	Phase 4 - Ozone Therapy in Endodontic Practice, in Vivo Study
NCT00970112	Excluded	O Uso de Dexametasona e Etoricoxibe Para a prevenção e Controle da Dor pós-operatória após Cirurgia Periodontal
NCT01063530	Excluded	Effects of DIammine SIlver Fluoride Placed Over Cervical Lesions of Permanent Teeth to Reduce Tooth Sensitivity
NCT01286298	Excluded	The Clinical Application of Diode Laser in Gingival Enlargement Related to Orthodontics
NCT01312194	Excluded	Periapical Healing After One or Two-visits to Endodontic Treatment in Adolescents Patients
NCT01346345	Excluded	Aesthetic Evaluation of Two Piece 3mm Implants for Single Tooth Replacement of Maxillary Laterals and Mandibular Incisors
NCT01402323	Excluded	A Prospective Split-mouth Designed Study on the Incidence of Gingival Clefts During Orthodontic Space Closure Into Recent or Healed Extraction Sockets.
NCT01503593	Excluded	Phase 3 Study of Using Combination of Bi Phasic Calcium Phosphate and Bu Phasic Calcium Sulphate During Extractions
NCT01547273	Excluded	The Effect of Bone and Connective Tissue Grafts on Facial Gingival Profile in Single Maxillary Anterior Immediate Implant Placement and Provisionalization: A 1-Year Prospective Study
NCT01547962	Excluded	A Pilot Clinical Trial to Assess the Safety and Efficacy of Gintuit (TM) (Allogeneic Cultured Keratinocytes and Fibroblasts in Bovine Collagen) in Establishing a Functional Zone of Attached Gingiva
NCT01591616	Excluded	A Phase 4 Pediatric Study to Assess the Pharmacokinetics and Safety of Oraqix Gel in Healthy Children and Adolescent Volunteers Following Tooth Extraction
NCT01628575	Excluded	Periodontally Accelerated Orthodontics - A Novel Technique For a Shortened Orthodontic Treatment With a Stable Result. A Clinical and Computerized Tomography Analysis
NCT01799187	Excluded	Randomized Controlled Research of Revascularization in Immature Permanent Teeth With Periapical Periodontitis
NCT01817413	Excluded	Revascularisation Versus Mineral Trioxide Aggregate in the Management of Non-Vital Immature Permanent Incisors in a Young Population: A Randomised Controlled Trial (Pilot Study)
NCT01821157	Excluded	Open-flap Versus Flapless Esthetic Crown Lengthening: 12-month Clinical Outcomes of a Randomized Controlled Clinical Trial
NCT01825772	Excluded	Clinical and Radiographic Outcomes of Dental Implant Therapy
NCT01827956	Excluded	Role of FCRIIIA and FCRIIA Receptor Polymorphisms in Cetuximab Activity Used in Palliative Treatment of Upper Aerodigestive Tract Tumours
NCT01870349	Excluded	Evaluation of Pro-inflammatory Mediators Around Astra Tech Dental Implant Abutments Following a Minimum of 6 Months of Clinical Function
NCT01899131	Excluded	A Prospective Clinical and Radiographic Assessment on New Platform-switched Laser Lok Implants
NCT01904552	Excluded	Influence of Apical Periodontitis on the Accuracy of Three Electronic Root Canal Length Measurement Devices: An In Vivo Study
NCT01944267	Excluded	Apically Positioned Flap, Free Gingival Graft and Apically Positioned Flap With Collagen Matrix Around Dental Implants
NCT01986959	Excluded	Periodontal Dressing After Surgical Crown Lengthening: A Randomized Clinical Trial
NCT02017886	Excluded	Salivary IgA Response to Dietary Supplementation of Lactobacillus Reuteri
NCT02027597	Excluded	Improving Oral Health With Serious Games
NCT02113137	Excluded	Effects of Various Oral Hygiene Procedures on the Reduction of Oral Malodor in Periodontally Healthy Patients
NCT02132546	Excluded	Spectrophotometric Evaluation of Chlorhexidine Pigmentations After Periodontal Flap Surgery: a Prospective Randomized Clinical Trial
NCT02143375	Excluded	Efficacy of810 nm Diode Laser on Gingival Pigmentation
NCT02174198	Excluded	The Esthetic Effect of Bio-OSS Collagen® on the Mid-facial Gingival Dimensions When Placed Into Gaps Between 3i® Implants Placed Into Fresh Extraction Sockets and the Labial Plate of Bone
NCT02196740	Excluded	Randomized Controlled Research of the Application of Triple Antibiotic Paste in Primary Teeth With
NCT02202304	Excluded	The Effects of Chlorhexidine/Thymol Varnish on the Abutment Teeth in Partial Denture Wearers.
NCT02221557	Excluded	Clinical and Histomorphometric Evaluation of Beta-tricalcium Phosphate/Polylactide Bone Substitute for Socket Preservation
NCT02276586	Excluded	Prospective Clinical Evaluation of Periodontal Response to Different Prosthetic Margin Design
NCT02309125	Excluded	Split Mouth Clinical Study to Compare the Secondary Stability of Dental Implants Using Immediate Gradual Loading Versus Early Loading Protocol in Posterior Maxilla. A Randomized Controlled Trial
NCT02315794	Excluded	Aesthetic Outcomes of Single Tooth Implant-supported Restorations Using Metal Ceramic Restorations With Either Zirconia or Titanium Abutments: A Randomized Controlled Clinical Study
NCT02343796	Excluded	Clinical Follow-up of Direct/Indirect Bridge, Orthodontic and Periodontal Splint Applications by Using Fibre-reinforced Composite.
NCT02352038	Excluded	Randomized Controlled Trial of a Novel Laser-aided Orthodontic-periodontal Treatment Strategy
NCT02359760	Excluded	Assessment of Piezoelectric Periodontal Surgery Effects on Orthodontic Treatment: a Prospective Pilot Study
NCT02369562	Excluded	A Retrospective Analysis of Failures/Complications With Oral Implants
NCT02374216	Excluded	A Prospective Analysis of Failures/Complications With Oral Implants
NCT02416700	Excluded	Evaluation of Peri Implant Bone Loss of Immediately Loaded Versus Conventionally Loaded Implants With a Single Prosthesis: A Randomized and Clinical Study Split-mouth Experimental Design

	NCT02423473	Excluded	Composite Resin Plus Connective Tissue Graft to Treat Gingival Recession Associated With Non-carious Cervical Lesion. Randomized Clinical Trial
1	NCT02435706	Excluded	Esthetic Outcomes of Single Immediate Implant Placement With Immediate Restoration Performed With Two Surgical Techniques
2	NCT02436525	Excluded	Microbial and Periodontal Changes Associated With Conventional Versus Self Ligating Brackets
3	CTRI/2008/091/000193	Excluded	An invivo study to evaluate the role of curcumin in oral pre-malignant lesions and gingivo-buccal cancers
4 5	CTRI/2011/04/001671	Excluded	Comparative evaluation of the effect of custom made anatomic healing abutment on papillary morphology, epithelial cuff formation around the implant and its emergence profile with that of conventional type of healing abutment - CustomHealingAbutment
6 7	CTRI/2011/08/001932	Excluded	Does application of oral chlorhexidine decrease the incidence of ventilator associated pneumonia in neonates: A Randomized controlled trial
8	CTRI/2012/03/002488	Excluded	Effects of low intensity laser therapy on the rate of orthodontic tooth movement :A clinical trial
9	CTRI/2013/11/004176	Excluded	Comparative evaluation of AlGaInAs diode laser and 5% sodium fluoride varnish in treatment of dentinal hypersensitivity in treated mild to moderate chronic periodontitis: a randomized controlled clinical trial
10	CTRI/2014/01/004333	Excluded	Single versus two visit pulpectomy treatment for primary teeth with apical periodontitis: a double blind, parallel group, randomized controlled trial.
11 12	CTRI/2014/03/004488	Excluded	Coronally advanced flap with a periosteal pedicle graft (PPG) VS sub epithelial connective tissue graft for root coverage procedures: a comparative analysis
13	CTRI/2014/07/004779	Excluded	Clinical Evaluation of CAD CAM fabricated Longterm provisional fixed partial dentures - A Randomised clinical trial
14	CTRI/2014/08/004850	Excluded	Effectiveness of Precaution Adoption Process Model as a behaviour change tool among Mothers of Children with Early Childhood Caries
15 16	CTRI/2014/09/004968	Excluded	Assessment of Yogic Relaxation Techniques for its Anxiolytic Effects in Patients Requiring Dental Surgery: AProspective, Randomized Controlled Study
17	CTRI/2014/09/005002	Excluded	Understanding the genetic basis of chemotherapy resistance in gingivobuccal squamous cell carcinoma (GSCC)
18	CTRI/2014/09/005031	Excluded	Antimicrobial action of Traditional Medicinal plant extracts on Aggregatibacter actinomycetemcomitans Isolated from Orodental Infections in the Suburbs of Mangalore and its Molecular Analysis.
19	CTRI/2015/02/005543	Excluded	Effect of hydrophobic sealant coating on plaque accumulation, gingival health, mutans streptococci count and caries occurrence of teeth banded/ bonded for fixed orthodontic therapy- A split mouth study in pediatric patients
20 21	CTRI/2015/04/005689	Excluded	To evaluate the efficacy of surgical stripping and diode laser techniques for gingival depigmentation. A clinical and histological study.
22	CTRI/2015/04/005709	Excluded	Development and evluation of an Oral care protocol on chemotherapy and radiation therapy induced oral complications in cancer patients: single blind, randomized clinical trialâ??
23 24	EUCTR2005-000973-24-IT	Excluded	Nimesulide spray in the treatment of simpthomatic inflammation with pain of the oral cavity (faringitis, stomatitis, pharyngitis) pre e post dental extraction. Randomised controlled vs active drug (Froben).
25 26	EUCTR2005-001623-11-HU	Excluded	Docetaxellel bövített standard 5-fluorouracil plusz ciszplatin alapú neoadjuváns kemoterápia plusz ciszplatin alapú radiokemoterápia összehasonlítása a standard ciszplatin alapú radiokemoterápiával lokoregionalisan elorehaladott (III-IV. stádiumú) fej-nyaki laphámrákok esetében. II. fázisú randomizált klinikai vizsgálat.
27 28	EUCTR2005-001885-14-BE	Excluded	A phase II, randomised, double blind, matched pair, controlled study to assess the safety and efficacy of Henogen recombinant soluble human tissue factor (rshTF) on the mandible bone consolidation and gingival cicatrisation in adults patients requiring orthognathic surgery - Henogen's rshTF for mandible bone consolidation and gingival cicatrisation
29 30 31	EUCTR2006-005788-24-CZ	Excluded	A Randomised, Double-Blind, Single dose, One-Day Early Administration, Multicentre Study comparing the Efficacy and Safety of Acyclovir Lauriad 50 mg muco-adhesive buccal tablet to matching Placebo, in the Treatment of Herpes Labialis in Immunocompetent Patients - LIP
32 33	EUCTR2009-014870-16-FR	Excluded	A phase II, multicentre, randomized, double-blind, placebo-controlled study comparing the efficacy and safety of Clonidine Lauriad® 50 µg and 100 µg mucoadhesive buccal tablet (MBT) applied once daily in patients to those of placebo in the prevention and treatment of chemoradion therapy-induced oral mucositis in patients with head and neck cancer
34 35	EUCTR2013-005305-31-SE	Excluded	Ice as topical anaesthesia before injection in the oral mucosa –a randomized unblinded cross-over study in adolescents. Comparison between ice and lidokain gel 5%. - Topical anaesthesia with ice in dentistry
36 37	DRKS00003127	Excluded	Oromucosal Delivery of Insulin: "Proof of Concept" Using a Mucoadhesive Wafer Dosage Form Containing Insulin. A single-center study with two parts: Part 1 comparing lingual/palatal versus gingival insulin wafer administration according to an open-label, parallel-group design, and, on condition that satisfactory exposure has been achieved, Part 2 investigating positive and negative control according to a double blind, crossover design. PART 2
38	DRKS00004248	Excluded	Pilot Study - Early space closure for prevention of gingival invaginations
39	DRKS00005596	Excluded	Psychophysiological effects of stress management in patients with gingivitis as well as in patients with gingivitis and additional atopic dermatitis - SBT
40	DRKS00007946	Excluded	Determination of factors influencing the bone resorption after dental implantation in a partly toothed dentition.
41	IRCT138709051081N2	Excluded	Analgesic Efficacy of celecoxib Versus Prednisolone For the Prevention and Control of Pain after Periodontal Surgery. -
42	IRCT138711061585N1	Excluded	Evaluation of clinical success, root resorption and periodontal changes in molars after intrusion using miniscrew.
43	IRCT138901243690N1	Excluded	The effect of gingival fiberotomy by Nd:YAG laser on Forced tooth eruption for clinical crown lengthening -
44	IRCT138902053795N1	Excluded	Evaluation of tooth staining by use of polyvinylpyrrolidone (pvp) in composition with Chlorhexidine -
45	IRCT138902201601N3	Excluded	Evaluation of success in lateral ridge augmentation with autogenous bone graft using trephine drills
46	IRCT138903264196N1	Excluded	Comparison of clinical and antimicrobial efficacy of 2% IKI and 2.5% sodium hypochlorite irrigants in infected root canals- An in vivo study -
47	IRCT201008114547N1	Excluded	The role of prophylactic ibuprofen and N-acetylcysteine on the levels of TNF- a and IL-6 in chronic periapical lesions -
48	IRCT201010265030N1	Excluded	Clinical evaluation of gingival condition around the implants in one implant retained mandibular overdentures and two implant retained mandibular overdentures with immediate loading in anterior mandible. -
49	IRCT201011085141N1	Excluded	Effect of Premedication of Ibuprofen and Dexamethasone on the Success of Inferior Alveolar Nerve Block for Teeth with Irreversible Pulpitis -
50	IRCT201110196443N2	Excluded	Comparison of the analgesic effect of odontopaste and a medicament with similar formulation in pain control after emergency treatment in the patients with lower posterior tooth pain - AES
51 52	IRCT201202018898N1	Excluded	A comparison of osteobiol gen_os with normal healing extraction socket in quality and quantity of bone formation for implant placement in partial edentulous patients - -
53	IRCT201202209085N1	Excluded	Effect of CO2 laser on demineralization resistance of enamel surface around orthodontic brackets(an in vivo study) -
54	IRCT201203047949N2	Excluded	Evaluation of the effects of thelower third molar surgery with use of demineralized bone powder on theperiodontal indexes on the surface of the second lower molar -
55	IRCT201205058242N2	Excluded	Effect of thricholoracetic-acid hydro gel on gingival margin location in cervical-composite restorations in the 20 to 40 years old pateints -
56 57	IRCT201205279882N1	Excluded	Comparison of periodontal ligament injection and inferior alveolar nerve block in mandibular primary molars pulpotomy: A randomized control trial -
58	IRCT201206121150N5	Excluded	The Effect of Low-Level Laser Therapy on Patient's Pain and Healing of Palatal Donor Site Following Free Gingival Graft -
59	IRCT2013011510935N2	Excluded	Clinical trial of comparison of heart rate and blood pressure changes due to administration of anesthesia agent with and without vasoconstrictor in healthy subjects. -
60	IRCT2013022512594N1	Excluded	Comparison of Recurrent Rate of Gingival Pigmentation after Treatment by Liquid Nitrogen and cryoprob in 18 Months Follow up in people who have anterior physiologic gingival pigmentation -

IRCT2013031712832N1	Excluded	Histological Evaluation of Pulp Tissue Following Direct Pulp Capping with Propolis and Dycal -
IRCT201304121760N22	Excluded	Comparison of analgesic effect of Acetaminophen and Acetaminophen Codeine with caffeine in control of control preapical periodontitis pain -
IRCT201305226443N3	Excluded	Comparision of the size of the upper anterior teeth periapical lesions between CHX %2 and NAOCL %5/25 by the radiography at 1 year after root canal therapy - ARS
IRCT201306121760N24	Excluded	Comparison of analgesic effect - the combination of acetaminophen and ibuprofen in the presence and absence of caffeine on pain after root canal treatment -
IRCT201307104731N12	Excluded	Pain releif effect of intravenous acetaminophen and pethidine in maxillofacial surgery
IRCT2013072414142N1	Excluded	Comparative evaluation of papilla reconstruction between adjacent implants using bone and connective tissue graft in patients referring to Periodontics Department of Hamadan School of Dentistry -
IRCT2013091012487N3	Excluded	Comparison of gingival depigmentation by open and closed cryosurgery in an Iranian population: A clinical trial -
IRCT2013121012487N4	Excluded	Comparison of Using Diode Laser or Abrasion in Gingival Depigmentation in an Iranian Population: A Clinical Trial -
IRCT2014052017781N1	Excluded	Compare of influence different two suture techeniques on periodontal healh of the mandibular second molars after extraction of impact third molar - Impact third molar
IRCT2014083118981N1	Excluded	5 year clinical evaluation of indirect full ceramic restorations(CEREC) - CAD/CAM
IRCT201502169014N55	Excluded	Antibacterial effect of sodium hypochlorite 6% plus chlorhexidine 0.2% and calcium hydroxide powder versus sodium hypochlorite 6% alone on the endodontic lesion in patients with apical periodontitis: a double blind randomized clinical trial
ISRCTN26302774	Excluded	Comparison of tooth replacement strategies for partially dentate older patients in the Republic of Ireland: a randomised controlled clinical trial
ISRCTN46246539	Excluded	Exposure of Palatal Canines: Cover-plate vs Periodontal Dressing - a Randomised Clinical Trial.
ISRCTN47483728	Excluded	Pulp molecular and gingival crevicular fluid (GCF) enzymological profiles during orthodontic treatment
ISRCTN55062915	Excluded	Efficacy of Anterior Middle Superior Alveolar nerve block (AMSA) versus Infra Orbital Nerve Block (IONB) for dental pulp and soft tissue anaesthesia in the anterior maxilla
ISRCTN56613406	Excluded	Self-ligating brackets and elastomeric rings - a comparison of orthodontic ligation techniques on patient oral hygiene and microbial colonization
ISRCTN67306656	Excluded	Long-term outcome and post-operative pain associated with chemo-mechanical root canal debridement using a manual-dynamic irrigation protocol in teeth associated with apical periodontitis: a randomised controlled trial
ISRCTN80708834	Excluded	Assessment of controlled release buccal inserts containing pilocarpine hydrochloride: a multi-centre, double-blind, placebo-controlled, randomised, cross-over study in patients diagnosed with Sjögren's Syndrome
JPRN-UMIN000002283	Excluded	A chemical on the antimicrobial prevention during tooth extraction in the patients with oral anti coagulation.
JPRN-UMIN000002607	Excluded	Clinical trial of electro-magnetic wave treatment for periapical periodontitis designed to disinfect and accelerate healing
JPRN-UMIN000005067	Excluded	Study of pain control for dental treatment by the density of diphenhydramine difference
JPRN-UMIN000007325	Excluded	The Effect of Gingival Melanin Depigmentaion by Dental Lazer Treatment.
JPRN-UMIN000008645	Excluded	Safety evaluation of a self-assembling peptide gel for the application as a hemostatic material in dentistry
JPRN-UMIN000009265	Excluded	The efficacy of azithromycin for the treatment of nonalcoholic steatohepatitis accompanied with Porphyromonas.gingivalis infection in the liver-Randomized controlled trial
JPRN-UMIN000009805	Excluded	Development of new topical anesthetic method -the study of the starch wafer method-
JPRN-UMIN000011286	Excluded	Safety investigations for the bone regenerative medicine using growth factors secreted from the patients' own stem cells
JPRN-UMIN000011290	Excluded	Bone regenerative medicine using allogeneic bone marrow derived mesenchymal stem cells secretome
JPRN-UMIN000011982	Excluded	A randomized cross-over study on the inhibitory effect of rice peptide on plaque formation
JPRN-UMIN000012236	Excluded	Periapical surgery using dental microscope based on diagnosis with CBCT
JPRN-UMIN000012794	Excluded	Study of the application of Igusa water extraction for mouse wash
JPRN-UMIN000013162	Excluded	Change of oral microbial composition by zinc ion
JPRN-UMIN000016057	Excluded	Effect of plaque removal on implant abutment using the ultrasonic water flow system
JPRN-UMIN000017088	Excluded	Effect of music sedation on psychological stress due to less invasive conservative dental treatment in patients with dental anxiety: Evaluation by autonomic nervous activity using heart rate variability
NTR2719	Excluded	Introduction of the ART technique in Upper Egypt: The influence of a special training program for general dental practitioners.
NTR3815	Excluded	Influence of thin and thick biotype on aesthetic outcome and possible manipulation of the biotype to enhance aesthetic result: A one-year randomized controlled clinical trial. - Influence and manipulation of the gingival biotype on aesthetic outcome
NTR4353	Excluded	The effects of radioiodine treatment on dental caries incidence in patients with differentiated thyroid carcinoma - N/A
NTR599	Excluded	The effect of continuation of anti-platelet agents on bleeding complications after dento-alveolar surgical procedures. - BLACK
RBR-25rc6q	Excluded	Colour stability of Photoactivated and Dual Resin Cements
RBR-6xsb5h	Excluded	The Effect of Low Level Laser Therapy in the Modulation of the Inflammatory Process After Surgery

Prefix legend	
ACTRN: Australian New Zealand Clinical Trials Registry	IRCT: Iranian Registry of Clinical Trials
ChiCTR: Chinese Clinical Trial Register	ISRCTN: International Standard Randomised Controlled Trial Number (International)
NCT: ClinicalTrials.gov (USA)	JPRN: Japan Primary Registries Network
KCT: Clinical Research Information Service (Korea)	NTR: Netherlands Trials Register
CTRI: Clinical Trials Registry (India)	RBR: Registro Brasileiro de Ensaios Clinicos (Brazil)
EUCTR: European Union Clinical Trials Register	SLCTR: Sri Lanka Clinical Trials Registry
DRKS: German Clinical Trials Register	TCTR: Thai Clinical Trials Registry

MeSH term	A	B1	B2
Cardiovascular Diseases			
Cardiovascular Diseases	18	3	9
Arteriosclerosis	1	0	4
Atherosclerosis	1	0	2
Peripheral Arterial Disease	1	0	0
Prehypertension	1	0	0
Hypertension	1	0	1
Coronary Disease	3	0	2
Coronary Artery Disease	0	0	1
Heart Diseases	3	1	2
Nutritional & Metabolic Diseases			
Diabetes Mellitus	46	12	14
Prediabetic State	2	0	0
Diabetes Mellitus. Type 2	36	7	6
Glucose Metabolism Disorders	51	12	17
Hyperlipidemias	1	0	0
Metabolic Syndrome X	2	0	2
Vitamin D Deficiency	0	1	0
Obesity	1	1	5
Musculoskeletal Diseases			
Arthritis. Rheumatoid	7	3	5
Sjogren's Syndrome	0	0	1
Spondylitis. Ankylosing	0	0	1
Osteoarthritis	0	0	1
Bone Diseases. Metabolic	0	2	3
Osteoporosis	0	1	2
Respiratory Tract Diseases			
Pneumonia. Bacterial	0	2	0
Respiratory Tract Infections	1	2	0
Pulmonary Disease. Chronic Obstructive	1	0	0
Respiratory Insufficiency	1	0	0
Respiratory Tract Diseases	3	3	0
Female Urogenital Diseases & Pregnancy Complications			
Pre-Eclampsia	1	0	1
Premature Birth	6	0	2
Pregnancy Complications	13	0	6
Renal Insufficiency. Chronic 2/2	8	0	1
Kidney Diseases 2/2	10	0	2
Male Urogenital Diseases			
Infertility. Male	0	0	1
Erectile Dysfunction	0	0	1
Renal Insufficiency. Chronic 1/2	8	0	1
Kidney Diseases 1/2	10	0	2
Pathological Conditions, Signs & Symptoms			
Genetic Predisposition to Disease	0	0	6
Yin Deficiency	1	0	0
Inflammation	19	2	8
Dyspepsia	1	0	0
Neoplasms			
Head and Neck Neoplasms	0	1	1
Breast Neoplasms	0	0	1
Neoplasms	0	2	4
Bacterial Infections & Mycoses			
Endotoxemia	1	0	1
Bacteremia	2	1	1
Virus Diseases			
Epstein-Barr Virus Infections	0	1	1
HIV Infections	0	0	3
Digestive System Diseases			
Gastroesophageal Reflux	0	0	1
Non-alcoholic Fatty Liver Disease	1	0	0
Nervous System Diseases			
Dementia	1	0	1
Alzheimer Disease	1	0	0
Reproductive & Urinary Physiological Phenomena			
Menopause	0	1	0
Pregnancy	0	4	1
Hemic & Lymphatic Diseases			
Anemia	1	0	0
Skin & Connective Tissue Diseases			
Lupus Erythematosus. Systemic	1	0	0
Immune System Diseases			
Immune System Diseases	8	3	9
Physiological Phenomena			
Nutritional Status	1	2	2
Immune System Phenomena			
Transplantation Immunology	0	0	1

Supplementary File S4

Systemic conditions that have been hypothesized to be linked with periodontal diseases, after labelling as MeSH terms.

A complete list is provided for the 57 systemic conditions that have been hypothesized to be related to periodontal diseases, along with the corresponding number of registration records in Categories A, B1 and B2. Colour code refers to the one chosen in Figures 1, 2 and 4.

Abbreviations: A - Periodontal intervention to improve (or prevent) a systemic condition; B1 - Intervention for a better understanding of the links between oral and overall health; B2 - Observational study of periodontal disease and a systemic condition.

Dear Editorial team of the Journal of Clinical Periodontology,

We are very grateful for the high quality of reviewing our manuscript received and the many pieces of advice on how to improve its quality and readability.

Our responses to the reviewers are noted point by point below. We give our explanation in response to each comment, together with the changes made. These changes are printed in red, both here and in the manuscript.

Page 4, line 8: replace "relative importance" with "evolution"
Page 4, line 26: grammar "deals"
Page 5, line 12: replace "describe" with " catalogue" (or "tabulate") (you do not describe the disease itself)
Page 6, line 15 and throughout the manuscript: citations in alphabetical order, style JCP
Page 6, line 19: add comma behind reference
Page 6, line 28: add reference Schenkein and Loos 2013, also from workshop EFP/AAP
Page 6, line 32: delete "As reported in 2012 by" and start "The" with capital
Page 6, line 34: replace "during" with "organized"; after "diseases," add "and reviewed"
Page 6, line 37: replace "have been associated" with "in association"
Page 7, line 5: add comma behind "(2013)"
Page 7, line 23: grammar "agrees"
Page 7, line 25: replace "worsening" with " severity"
Page 8, line 5: replace "classified" with " catalogued"
Page 8, line14: replace "describe" with " catalogue" (or "tabulate")
Page 8, line 19: replace "relative importance" with " evolution"
Page 8, line 44: provide here website address
Page 12, line 10: spelling "assignments"
Page 13, line 45: no enter behind ".... field of research.". Start next sentence ("Some characteristics ....) at a new line.

All these issues have been corrected.

Page 13, lines 52-57: Rewrite: you mean: mainly from Asia? Clarify for all or for periodontal medicine?

We have rewritten as follows (Page 10, line 15): Registration records were mainly from Asia, Europe and North America: 51.4%, 23.9% and 14.8% for periodontal dentistry, 42.5%, 20.8% and 18.1% for periodontal medicine, respectively.

Page 14, line 40: change sequence as follows: "Diabetes Mellitus and Rheumatoid Arthritis"
Page 14, line 47: replace " been hypothesized" with " appeared in the tabulation"
Page 16, line 16: add comma behind "registers"; grammar "deals"
Page 16, line 28: only put "(2013)" between parenthesis
Page 17, line 14: no enter behind "(Niederman et al., 2002)." In general, wrap your text and make robust paragraphs
Page 18, line 34: replace " has" with "was" and "describe" with " tabulate"
Page 18, line 50: replace "by Nogueira Filho and Tenenbaum" with " previously"
Page 19, line 12: add "often" behind ".... studies are"; replace "practicable" with "feasible"
Page 19, line16: replace "of 16%" with "of the registered trials to be observational studies (16%)"

Page 19, lines 23-35: delete paragraph. This is your opinion on a broad topic that is not investigated by you – workshop EFP made recommendations.
Page 20, line 14: replace "still lacking" with "hardly explored"

All these issues have been corrected.

Page 19, lines 36-54: rewrite this paragraph – really focusing on your own research here: registrations have reported types of intervention and a lack of patient centered outcomes, this can then be discussed here only in that context.

As suggested, we have modified the following paragraph (Page 14, line 28): Although periodontal treatment modalities fall outside the scope of the present work, the screening of trial registers has revealed great diversity in this field (Drisko, 2001, Bader, 2010). ~~Evidence about which types of treatment may be helpful and which may be harmful, in whom, and why, is needed to guide practice and policy (Vohra and Boon, 2015).~~ We did not notice alternative methods of interventional research, such as n-of-1 trials (Lillie et al., 2011), a methodology that has never been investigated in periodontal research as far as we know. Finally, we noted a lack of patient-centred outcomes in RCTs, while this concept is of fundamental importance considering that whole-person care is the cornerstone of periodontal medicine.

Page 20, line 21: you could mention another example, published in the JCP: Hettne et al. 2007, JCP34:1016-1024

Thank you very much for this improvement. The modified text now reads (Page 14 and 15, line 58 and 3) : Data mining and Knowledge Discovery in Databases (KDD) on periodontal research (Hettne et al., 2007), as initiated in the present analysis, would highlight the necessity for new research directions (Piatetsky-Shapiro and Frawley, 1991, Fayyad U et al., 1996), and would also identify gaps in research. The combination of bioinformatics tools and medical data would result in significant advances in the understanding of pathophysiology and individual susceptibility (Hettne et al., 2007).

Page 20, line 38-45: delete this paragraph – see my previous comments.
Page 20, line 50: replace "this study" with "analyses of trial registers"
Page 20, line 52: replace "current periodontal research" with "periodontology"
Page 39, Figure 2: behind "hypothesized" add "in the registers"
Page 40, Figure 3: legend of A+B1+B2: change to an open square to offset it from the closed circles for C+D (this is more clear for the black + white copies); remove "beyond"
Page 46, line 6: add "the" before " possible"
Page 46: To be consistent – rewrite name of all studies in lower case, some are in capitals e.g. NCT01906450

All these issues have been corrected.

Page 22: improve the references to follow the style of the JCP: journal names in full

We apologize for this. All references have been modified.

Page 37: improve Table legend: something like: Listing of all trial registers and global/geographical areas and subdivision into types of research questions.

As suggested, we have modified the Table legend (Page 30):

Characteristics of registration records. The number of registered trials for periodontal dentistry and periodontal medicine is detailed by trial register and geographical area, and by median study size.

Page 39, Figure 2: please make sure all diseases are well readable in the figure. My black and white print for example did not allow for good representation. Perhaps white letters in dark colored compartments.

In order to improve this figure, we have provided a new figure 2 with bigger font size and lighter background colors (Page 27).

Page 41, Figure 4: improve/correct figure legend: the categories A, B1 and B2, and G are not visible on the graph

We apologize for this mistake. The legend has been modified (Page 29). This chord diagram represents the proportion of studies dealing with each included sub-branch of the MeSH classification "Diseases" [C] and "Phenomena and Processes" [G], linked to the respective start years of the studies (grouped in 4 periods: 1998-2005, 2006-2008, 2009-2011, 2012 and beyond).

Page 45, Figure: add in Figure the diseases like in Figure 4. Write them out

Supplementary File S2 has been entirely modified as suggested (Page 34).

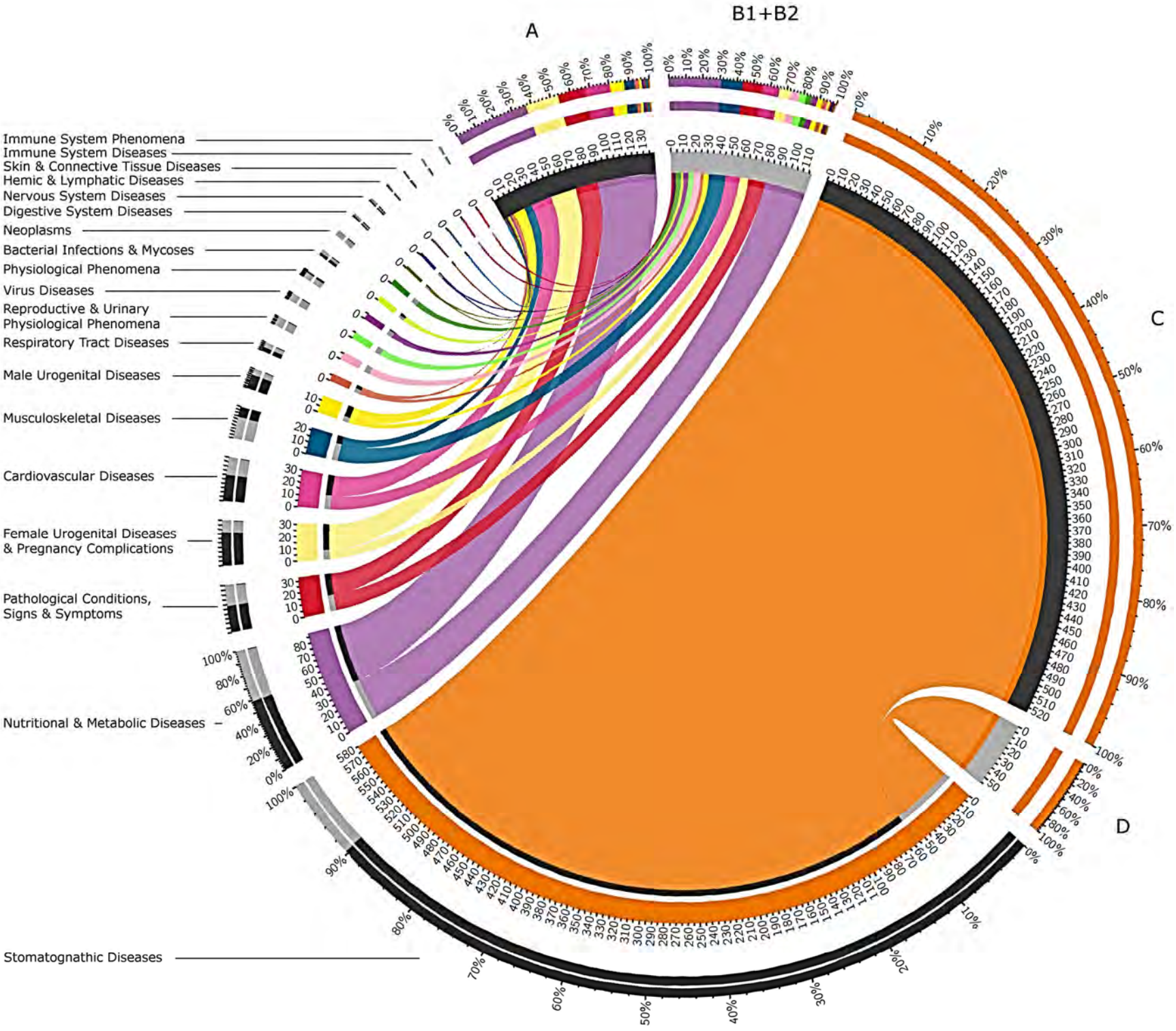
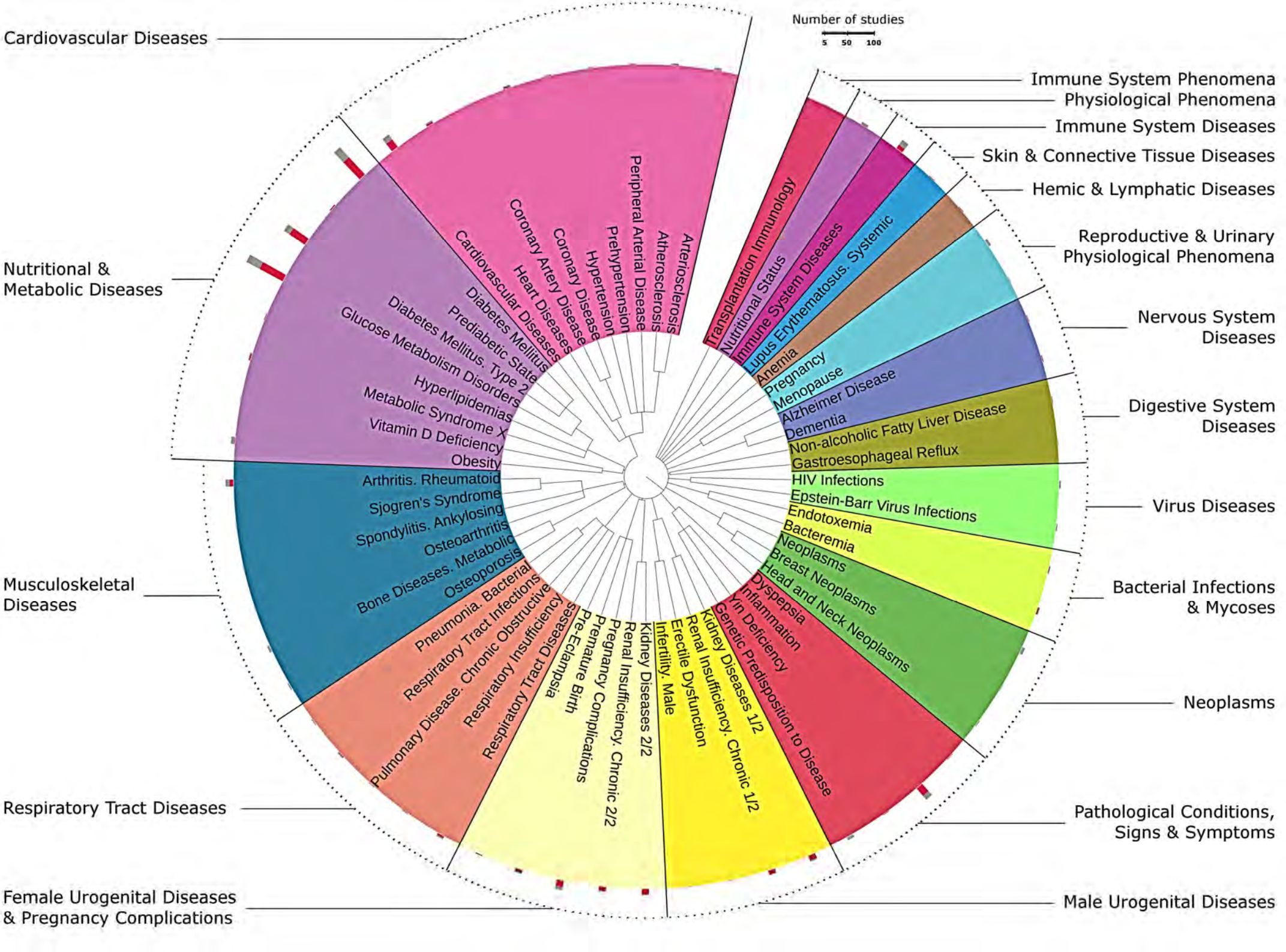

A
B1+B2
C
D
Immune System Phenomena
Immune System Diseases
Skin & Connective Tissue Diseases
Hemic & Lymphatic Diseases
Nervous System Diseases
Digestive System Diseases
Neoplasms
Bacterial Infections & Mycoses
Physiological Phenomena
Virus Diseases
Reproductive & Urinary Physiological Phenomena
Respiratory Tract Diseases
Male Urogenital Diseases
Musculoskeletal Diseases
Cardiovascular Diseases
Female Urogenital Diseases & Pregnancy Complications
Pathological Conditions, Signs & Symptoms
Nutritional & Metabolic Diseases
Stomatognathic Diseases

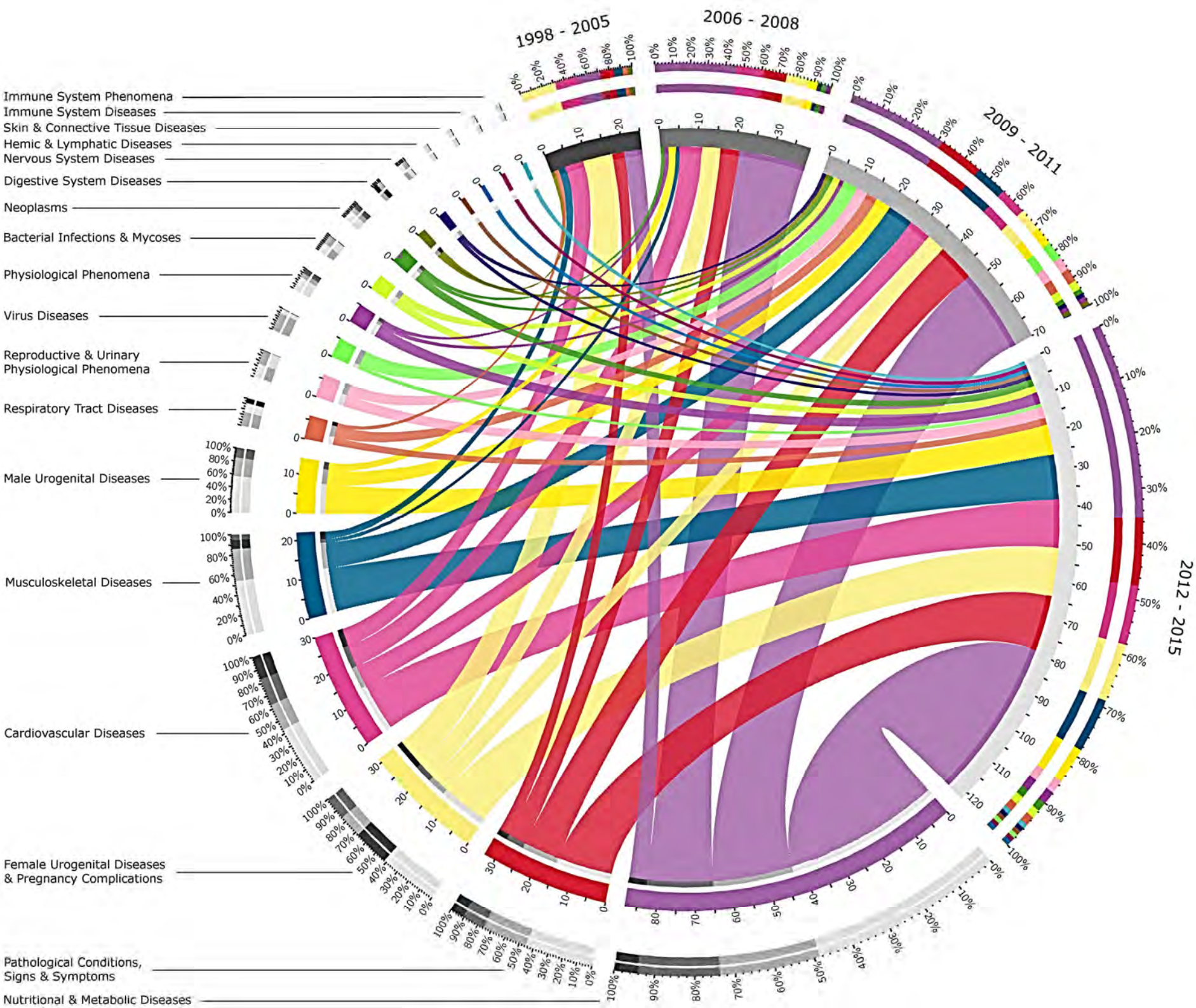
Figure 2

Cette représentation est un super arbre MeSH. Il permet de visualiser un ou plusieurs jeux de données en fonction d'un mot clef du MeSH.

La partie externe présente l'ensemble des mots clefs MeSH (taxons terminaux) classifiant les enregistrements des essais cliniques. Au bout de ces taxons se trouvent des histogrammes empilés donnant le nombre et la proportion d'études de type A (rouge, études interventionnelles avec causalité parodontite/ maladie systémique), ou B1+B2 (gris, interventionnelles ou observationnelles avec non causalité parodontite/maladie systémique).

La partie interne du diagramme présente l'arborescence du MeSH (i.e. sa complexité). Elle permet de voir à quel endroit du MeSH se situe le mot clef (taxon terminal) que l'on voit à l'extérieur du cercle, et de voir les différentes divisions qu'il y a entre ces branches. Par exemple, Glucose Metabolic Disorders (C18.452.394) est plus en amont de Diabetes, Mellitus (C18.452.394.750). Cela veut donc dire que dans l'histogramme de Glucose Metabolic Disorders se trouvent également les études de Diabetes, Mellitus.


Number of studies
5
50
100
Cardiovascular Diseases
Nutritional & Metabolic Diseases
Musculoskeletal Diseases
Respiratory Tract Diseases
Female Urogenital Diseases & Pregnancy Complications
Male Urogenital Diseases
Pathological Conditions, Signs & Symptoms
Neoplasms
Bacterial Infections & Mycoses
Virus Diseases
Digestive System Diseases
Nervous System Diseases
Reproductive & Urinary Physiological Phenomena
Hemic & Lymphatic Diseases
Skin & Connective Tissue Diseases
Immune System Diseases
Physiological Phenomena
Immune System Phenomena
Arteriosclerosis
Atherosclerosis
Peripheral Arterial Disease
Prehypertension
Hypertension
Coronary Disease
Coronary Artery Disease
Heart Diseases
Cardiovascular Diseases
Prediabetic State
Diabetes Mellitus
Diabetes Mellitus. Type 2
Glucose Metabolism Disorders
Hyperlipidemias
Metabolic Syndrome X
Vitamin D Deficiency
Obesity
Arthritis. Rheumatoid
Sjogren's Syndrome
Spondylitis. Ankylosing
Osteoarthritis
Bone Diseases. Metabolic
Osteoporosis
Pneumonia. Bacterial
Respiratory Tract Infections
Pulmonary Disease. Chronic Obstructive
Respiratory Insufficiency
Respiratory Tract Diseases
Pre-Eclampsia
Premature Birth
Pregnancy Complications
Pregnancy. Chronic 2/2
Renal Insufficiency. Chronic 2/2
Kidney Diseases 2/2
Infertility. Male
Erectile Dysfunction
Renal Insufficiency. Chronic 1/2
Kidney Diseases 1/2
Genetic Predisposition to Disease
Yin Deficiency
Inflammation
Dyspepsia
Head and Neck Neoplasms
Breast Neoplasms
Neoplasms
Bacteremia
Endotoxemia
Epstein-Barr Virus Infections
HIV Infections
Gastroesophageal Reflux
Non-alcoholic Fatty Liver Disease
Dementia
Alzheimer Disease
Menopause
Pregnancy
Anemia
Lupus Erythematosus. Systemic
Immune System Diseases
Nutritional Status
Transplantation Immunology


1998 - 2005
2006 - 2008
2009 - 2011
2012 - 2015
Immune System Phenomena
Immune System Diseases
Skin & Connective Tissue Diseases
Hemic & Lymphatic Diseases
Nervous System Diseases
Digestive System Diseases
Neoplasms
Bacterial Infections & Mycoses
Physiological Phenomena
Virus Diseases
Reproductive & Urinary Physiological Phenomena
Respiratory Tract Diseases
Male Urogenital Diseases
Musculoskeletal Diseases
Cardiovascular Diseases
Female Urogenital Diseases & Pregnancy Complications
Pathological Conditions, Signs & Symptoms
Nutritional & Metabolic Diseases

II.1.5.2 Principaux éléments de discussion et perspectives

Une grande variété de pathologies systémiques associées à la parodontite

Pour la première fois, cette étude a permis de cartographier et de cataloguer les pathologies systémiques qui pourraient être associées aux maladies parodontales. Bien que 57 pathologies systémiques aient été identifiées, leur nombre est certainement sous-estimé puisque les registres prennent moins en considération les études observationnelles et ne sont pas garants de l'intégralité des essais cliniques. Par exemple, nous n'avons pas retrouvé d'étude explorant le lien entre prostatite et parodontite même si cela a déjà été évoqué dans la littérature (40). Ceci nous conforte dans l'idée d'explorer maintenant la base de données PubMed ; le volume élevé de données dont nous pourrions disposer permettrait de dégager de nouvelles pistes d'investigation, notamment de nouvelles hypothèses de mécanismes physiopathologiques impliqués, de force d'association et de causalité (41).

CSMs, parodontite et pathologies générales

Puisqu'il s'agit d'enregistrements d'essais cliniques, il est nécessaire de ne pas faire d'amalgame entre identification de pathologies systémiques et leur potentielle association significative avec la parodontite. Néanmoins, puisque de nombreuses hypothèses sont formulées sur cette association (causale ou non causale), ce travail met en exergue la nécessité d'étudier le comportement des CSMs provenant de donneurs potentiellement pathologiques, et les différents facteurs pouvant influencer leur prise en charge pour de la thérapie cellulaire du parodonte. Ces facteurs pourraient être des facteurs techniques (i.e. modification du rendement de progéniteurs par gramme de tissu) ou des facteurs biologiques (i.e. modification des propriétés de différenciation, d'immunomodulation). Une meilleure connaissance de ces paramètres permettrait de mieux poser les indications de greffe cellulaire allogénique.

II.1.6 Parodontite et CSMs endogènes : hypothèses

Origine des CSMs impliquées dans la cicatrisation du parodonte profond

Des CSM (42, 43) ont été localisées chez l'homme au niveau du ligament parodontal, dans sa partie apicale, cervicale et inter-radiculaire de dents saines ou présentant des lésions parodontales (42). Ces cellules sont surtout retrouvées au niveau péri-vasculaire. Néanmoins,

la présence de CSMs dans les zones extravasculaires (et au niveau du cément) est plus importante au niveau de parodontes pathologiques ; ceci pourrait donc être dû à une migration, prolifération/différenciation de CSMs provenant des zones péri-vasculaires. En effet, les auteurs font l'hypothèse d'un passage des espaces endostéaux de l'os alvéolaire vers les zones péri-vasculaires du ligament puis vers les zones extravasculaires en réponse à un signal provenant des tissus lésés (42).

Des lésions parodontales crées chez la souris chimérique obtenue par reconstitution après irradiation par des cellules médullaires de souris transgéniques GFP+ ont permis d'objectiver que des progéniteurs provenant de la moelle osseuse étaient recrutés sur les sites pathologiques (44). Ceci signifierait donc que des progéniteurs médullaires, et potentiellement de l'os alvéolaire, seraient mobilisés lors de l'installation/évolution de la parodontite (45). La moelle osseuse agit comme un réservoir pour des populations multiples de progéniteurs qui peuvent être mobilisées dans la circulation périphérique après une lésion, même si ces mécanismes pourraient être facilement débordés (46).

Parmi les facteurs favorisant la migration des cellules au travers de l'endothélium, la chimiokine CXCL12 (SDF-1) et son récepteur le CXCR4, jouent un rôle majeur (47). Le récepteur CXCR4 est présent à la surface des ASCs et l'hypothèse de la migration des ASCs depuis le tissu adipeux vers le parodonte peut être évoquée (48). Les ASCs peuvent être mobilisés dans la circulation périphérique, même si le rôle de cette fonction n'est pas encore connu (49). Il s'agirait peut-être d'un rôle de soutien à la néovascularisation (50). Ainsi, en plus des progéniteurs intra-ligamentaires, de la moelle osseuse, la contribution de progéniteurs issus du tissu adipeux dans la régénération parodontale est donc une hypothèse crédible ; en particulier, un tissu adipeux plus proche de l'environnement parodontal, le corps adipeux de la bouche. Son étude serait donc intéressante pour tenter de répondre à ces questions.

Pour modéliser cette hypothèse, une greffe de tissu adipeux inguinal de souris GFP+ pourrait être réalisée au niveau du corps adipeux de la bouche ou au niveau abdominal de souris non GFP. Après avoir réalisé une lésion du parodonte, la distribution des cellules GFP+ pourrait-être évaluée. Une autre approche consisterait à disposer de souris génétiquement modifiées pour avoir exclusivement des ASCs marqués par GFP (ce modèle est en cours d'élaboration dans le laboratoire), ce qui permettrait de visualiser les ASCs ou cellules dérivées des ASCs à l'intérieur du parodonte de souris.

CSMs, conditions systémiques et régénération parodontale

La parodontite est la sixième complication du diabète (51) : les parodontites sont plus nombreuses et plus sévères, la réponse au traitement est moindre à long terme (52), et les délais de cicatrisation muqueuse sont plus élevés que chez les patients non diabétiques (53). Il est donc nécessaire de mieux comprendre le mécanisme de réparation chez ces patients pour mieux prévoir la réponse au traitement et les objectifs de régénération par thérapie cellulaire.

Les CSMs de patients diabétiques ont des propriétés de migration inférieures aux cellules de patients non diabétiques (54) : la « pression » intra-médullaire trop forte de SDF-1 chez ces patients pourrait être responsable d'un gradient chimiotactique défavorable, empêchant la migration des CSMs vers les tissus lésés (54). Les cellules progénitrices endothéliales (EPC) répondent aussi moins bien au gradient de SDF-1, pouvant limiter revascularisation et réparation endothéliale (55).

Un tel phénomène serait donc tout à fait envisageable au niveau parodontal, d'où la cicatrisation de moindre qualité. Il serait ainsi intéressant de corréler la réponse au traitement parodontal en fonction du nombre de CSMs circulantes, à la fois chez des patients sains et chez des patients atteints de pathologies systémiques. Une question similaire se pose chez des patients présentant d'autres pathologies systémiques, comme le lupus systémique où le SDF-1 est significativement augmenté au niveau du rein (56).

La thérapie cellulaire par CSMs permettrait de créer un gradient chimiotactique pour rétablir l'attractivité du site parodontal pour les progéniteurs, favorisant ainsi la régénération tissulaire. Il est donc fondamental de mieux comprendre l'influence des caractéristiques médicales et sociodémographiques des donneurs sur le comportement des CSMs (dont comportement migratoire).

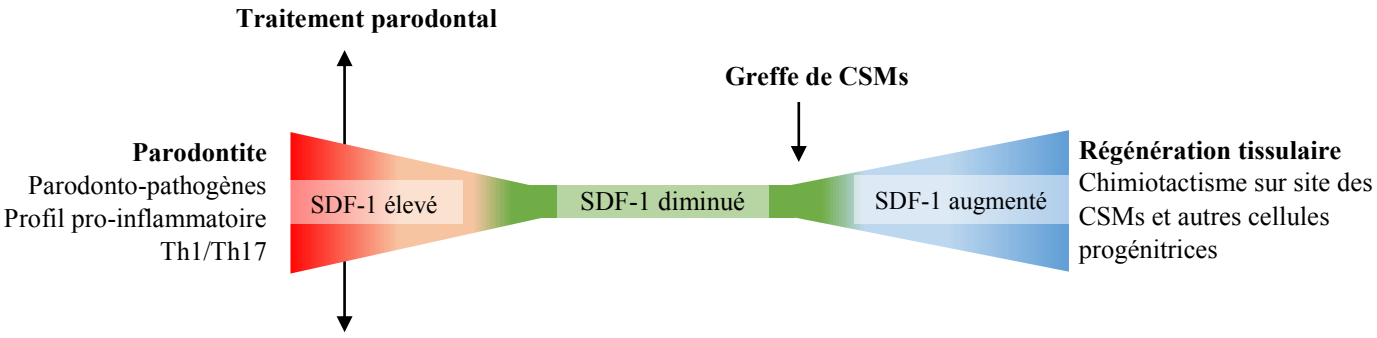


Figure II-4 : Le signal SDF-1 est augmenté au niveau du fluide gingival et des tissus gingivaux de patients atteints de parodontite (44). Cette augmentation de signal est liée à un recrutement des cellules de défense au niveau des sites inflammatoires (57, 58). Mais ce signal revient à un niveau basal après traitement parodontal (57). La greffe de CSMs au niveau de sites parodontaux traités permettrait d'augmenter le gradient chimiotactique et le recrutement des progéniteurs locaux et circulants sur le site parodontal (59).

CSM et immunité

Bien qu'il soit difficile de reproduire ces effets *in-vitro*, la capacité des CSMs à adopter un phénotype différent en fonction du contexte inflammatoire de l'environnement, permet d'envisager une application de thérapie cellulaire par CSM pour des pathologies de la réponse immunitaire allogénique ou autologue, comme la maladie du greffon contre l'hôte (GvHD ou *graft-vs-host disease*) ou les maladies auto-immunes (60). Ces cellules peuvent interagir directement ou indirectement avec le système immunitaire inné et adaptatif (60). Les CSMs ont un effet immunomodulateur varié avec une action sur les cellules dendritiques, lymphocytes T CD4+/CD8+, lymphocytes B, NK, monocytes macrophages et neutrophiles (61). Les CSMs vont convertir les macrophages du profil M1 (pro-inflammatoire) en M2 (anti-inflammatoire) et établir un profil lymphocytaire TH1/TH2 favorable avec une augmentation des lymphocytes T régulateurs (61).

Cet effet immunosuppresseur/immunorégulateur serait lié à la sécrétion de facteurs solubles tels que des cytokines (i.e. IL-10), prostaglandine E2 (PGE2), monoxyde d'azote (NO) ou via l'indoléamine oxydase (IDO, convertissant le tryptophane en kynurénine)(62).

L'environnement serait responsable d'une polarisation des CSMs en profil CSM1/CSM2 (selon le même principe que TH1/TH2 ou M1/M2) (60). L'activation de la voie des TLR-4 engagerait les CSMs vers un profil pro-inflammatoire alors que l'activation de la voie des TLR-3 vers un profil anti-inflammatoire. Dans un contexte pro-inflammatoire (avec des hauts niveaux d'IFNγ et de TNFα, les CSMs adopteraient un profil CSM2 immunosuppresseur en sécrétant par

exemple IDO, PGE2, NO ainsi que TGF-β, favorisant l'émergence des lymphocytes T régulateurs. Les CSMs aideraient donc au rétablissement du déroulement temporel de la réponse immuno-inflammatoire, en favorisant soit l'aspect pro ou anti-inflammatoire, promouvant d'un côté la réponse de défense, prévenant de l'autre une réponse excessive pour éviter les lésions tissulaires et permettre la régénération (60). Il ne semble pas y avoir de différence majeure au niveau immunomodulation par BM-MSC et ASC : sécrétion de médiateurs, suppression de la réaction lymphocytaire mixte (63). Ainsi les effets immunomodulateurs des ASC ont été validés dans de nombreux modèles précliniques et/ou en clinique comme les maladies neurodégénératives, lésions de la moelle épinière, pathologies auto-immunes, maladie du greffon contre l'hôte (63).

Néanmoins, certaines pathologies systémiques (64), ou encore les conditions de stockage des CSM (65) (i.e. cryopréservation) modifieraient le comportement immunomodulateur des cellules. Ces paramètres doivent donc rentrer en ligne de compte dans la compréhension de la physiopathologie des maladies et de leur prise en charge par la greffe de CSM en fonction des potentialités cellulaires supposées et attendues.

II.1.7 Thérapeutique parodontale

Puisque les microorganismes impliqués dans la maladie parodontale sont associés en biofilms (10, 11), qui créent un environnement qui réduit la sensibilité aux antibiotiques et aux antiseptiques (10, 12), son débridement mécanique est une composante essentielle de la thérapie parodontale (66).

L'éducation du patient et sa motivation à l'hygiène orale est la base d'une approche préventive du soin parodontal, et l'élément indispensable dans une thérapeutique parodontale (67, 68). Le traitement conventionnel inclut les thérapeutiques chirurgicales et non chirurgicales, utilisées seules ou en association, afin de décontaminer le cément. Les thérapies non chirurgicales consistent en un débridement mécanique de la plaque et du tartre sous-gingival au moyen d'instruments ultrasoniques, soniques ou manuels et un polissage (67). Selon la sévérité clinique, basée en grande partie sur les lésions infra-osseuses résiduelles (69), les interventions chirurgicales peuvent être nécessaires afin de retirer le tartre résiduel dans les parties les plus profondes des poches parodontales ou afin de changer l'anatomie de l'environnement (70). Les antiseptiques (bains de bouche, dentifrices ou gels) et les antibiotiques (administrés de manière

systémique ou locale) sont souvent associés aux traitements parodontaux même si les micro-organismes parodontaux peuvent acquérir des résistances aux antibiotiques (71-73).

Néanmoins, environ 20% des patients répondent faiblement au traitement parodontal (74-78). Plusieurs hypothèses peuvent être évoquées pour expliquer cette susceptibilité individuelle: les patients réfractaires pourraient avoir des particularités cliniques comme un microbiote sous-gingival spécifique (hauts niveaux de pathogènes parodontaux ou augmentation de pathogènes non connus) ; ou une immunité déficiente (74, 75), ou les deux, ou encore un déficit de mobilisation des progéniteurs dans la circulation périphérique et/ou « d'homing », expliquant la moins bonne réponse régénérative.

Compte tenu des liens avec les pathologies systémiques et la qualité de vie, compte tenu des hypothèses physiopathologiques évoquées d'inflammation et d'infection, le traitement parodontal permet d'augmenter la durée de vie des dents sur l'arcade, améliorant la qualité de vie générale et orale des patients. L'obtention d'une régénération tissulaire doit s'entendre dans un contexte de résolution à long terme de l'infection et de l'inflammation.

II.1.8 La régénération du parodonte profond

Même si le compromis thérapeutique reste souvent la stabilisation de la pathologie, l'objectif final et optimal doit être la restitution *ad integrum* du parodonte profond, c'est-à-dire la régénération d'une attache conjonctive fonctionnelle avec un ligament parodontal, du cément et de l'os alvéolaire.

Plusieurs procédures à visée régénérative peuvent être utilisées seules ou en association, comme l'utilisation des protéines dérivées de la matrice de l'émail (79), la régénération tissulaire guidée (70) ou les greffes osseuses (79). Mais aucune de ces techniques n'est parfaitement prédictible et le pronostic reste encore aléatoire. L'environnement parodontal pathologique implique une contamination du cément par les bactéries et ses toxines, des altérations de surface liées à l'inflammation (80).

De nouveaux traitements devront donc tenir plus particulièrement compte de l'étiopathogénie et de la physiopathologie de la maladie parodontale. La régénération tissulaire doit impliquer le recrutement et la différenciation des cellules progénitrices (rendre le milieu propice à la réactivation des progéniteurs naturels *in situ*), la stimulation de la minéralisation et le contrôle de la sécrétion des protéines matricielles (80).

Comme nous l'envisagerons par la suite, l'utilisation des propriétés des CSMs en général et des ASCs en particulier pour la régénération parodontale est donc une option à considérer (81). Nous posons l'hypothèse qu'elles permettraient un retour durable à l'homéostasie tissulaire en prenant en charge à la fois la destruction tissulaire, les éléments microbiens, et l'inflammation chronique persistants souvent après le traitement, alors même que la symptomatologie clinique post thérapeutique revêt transitoirement des aspects compatibles avec la fonction. L'environnement est également dépendant de cofacteurs locaux et systémiques qu'il convient de prendre en considération, et qui pourraient avoir une influence sur le devenir des cellules greffées et l'effet régénératif.

II.2 Régénération parodontale chez l'animal

II.2.1 Article 4: "Mesenchymal stromal cells used for periodontal regeneration: a systematic review" avec Article 5: "Cell therapy of periodontium: from animal to human?"

Le travail présenté dans ce chapitre fait l'objet d'un article publié dans « Stem Cells and Translational Medicine » et d'un article publié dans « Frontiers in Craniofacial Biology ».

Contexte

La littérature scientifique fait état d'un nombre croissant d'expérimentations animales sur le bénéfice potentiel de l'utilisation des CSMs quant à l'objectif de régénération parodontale. Les revues de littérature nous offrent très certainement une vision trop optimiste du domaine, et des méthodes de synthèse basées sur des critères et stratégies de recherche préalablement définies, sont nécessaires.

L'approche de type revue systématique, doit-être par définition exhaustive au niveau de la recherche des informations, bénéficier de l'objectivité de l'examinateur et être reproductible. La synthèse des données peut être également couplée à une compilation statistique (méta-analyse) afin de dégager des sous-groupes de données. Parmi les différents types de revues systématiques, la revue exploratoire (« scoping study ») (82) a été choisie pour sa capacité à cartographier la littérature scientifique tout en mettant en évidence les manques en terme de connaissance. L'utilisation combinée à une analyse qualitative des contenus scientifiques permet de réfléchir à de nouvelles directions de recherche (83, 84). Dans le cas présent, l'approche suivie est principalement configurative, c'est-à-dire que les objectifs et critères de sélection ont progressivement évolués, de manière itérative lors de la sélection des articles.

L'objectif de cette revue exploratoire de la littérature est de quantifier de manière objective les éléments concernant l'efficacité et la sureté des procédures de régénération parodontale par CSMs, et d'identifier des éléments clefs permettant d'expliquer les différences observées entre les études.

Méthodologie

La recherche électronique s'est basée sur l'exploration de 8 bases de données, dont PubMed. Nous avons également effectué une recherche de littérature grise par l'intermédiaire du portail

de l'OMS (ICTRP) et des résumés de conférences internationales comme EUROPERIO et l'IADR (International Association for Dental Research). La dernière mise à jour a été effectuée le 25/08/2013. Toutes les études évaluant la régénération des tissus parodontaux profonds (os alvéolaire, cément et ligament) avec la mise en place d'un groupe témoin, ont été sélectionnées. Ont été considérées des variables centrées sur le patient et la société (coût, satisfaction ou qualité de vie), de sécurité (comme les effets indésirables), de régénération (évaluée par des méthodes cliniques, radiologiques ou histologiques) ou concernant l'environnement local (par l'exploration d'aspects inflammatoires ou bactériens).

Résultats

Sur les 2986 études évaluées, 56 études (45 animales et 11 humaines) ont été inclues. Cette revue systématique a montré que l'utilisation des CSMs est sure et efficace pour la régénération des tissus parodontaux mais qu'il est nécessaire de comprendre quelle est la combinaison la plus appropriée de source cellulaire et de biomatériau en fonction du cas clinique. Les modèles animaux utilisés sont également peu représentatifs de la physiopathologie des parodontites.

Articles

Concise Review: Mesenchymal Stromal Cells Used for Periodontal Regeneration: A Systematic Review

Paul Monsarrat, Jean-Noël Vergnes, Cathy Nabet, Michel Sixou, Malcolm L. Snead, Valérie Planat-Bénard, Louis Casteilla and Philippe Kémoun

Stem Cells Trans Med published online April 17, 2014

Published Ahead of Print on April 17, 2014 as 10.5966/sctm.2013-0183
The latest version is at http://stemcellstm.alphamedpress.org/cgi/doi/10.5966/sctm.2013-0183

STEM CELLS TRANSLATIONAL MEDICINE

TISSUE ENGINEERING AND REGENERATIVE MEDICINE

Concise Review: Mesenchymal Stromal Cells Used for Periodontal Regeneration: A Systematic Review

PAUL MONSARRAT,[a,b,c,d,e,f] JEAN-NOËL VERGNES,[e] CATHY NABET,[e] MICHEL SIXOU,[e] MALCOLM L. SNEAD,[g] VALÉRIE PLANAT-BÉNARD,[a,b,c,d] LOUIS CASTEILLA,[a,b,c,d] PHILIPPE KÉMOUN[f]

Key Words. Mesenchymal stromal cells • Stem cells • Periodontal diseases • Tissue engineering • Review • Systematic

[a]STROMALab, Toulouse, France; [b]Université de Toulouse, Toulouse, France; [c]INSERM, Toulouse, France; [d]Etablissement Français du Sang Pyrénées-Méditerranée, Toulouse, France; Departments of [e]Public Health and [f]Biology, Toulouse Faculty of Dentistry, Paul Sabatier University and Toulouse University Hospital, CHU de Toulouse, Toulouse, France; [g]Center for Craniofacial Molecular Biology, Herman Ostrow School of Dentistry, Center for Craniofacial Molecular Biology, University of Southern California, Los Angeles, California, USA

Correspondence: Philippe Kémoun, D.D.S., Ph.D., Faculté de Chirurgie Dentaire, 3, Chemin des Maraîchers, 31062 Cedex 09, Toulouse, France. Telephone: 33-620013738; E-Mail: philippe.kemoun@univ-tlse3.fr

Received October 9, 2013; accepted for publication February 3, 2014.

1066-5099/2014/$20.00/0

http://dx.doi.org/10.5966/sctm.2013-0183

ABSTRACT

Periodontitis is a chronic infectious disease of the soft and hard tissues supporting the teeth. Recent advances in regenerative medicine and stem cell biology have paved the way for periodontal tissue engineering. Mesenchymal stromal cells (MSCs) delivered in situ to periodontal defects may exert their effects at multiple levels, including neovascularization, immunomodulation, and tissue regeneration. This systematic review had two goals: (a) to objectively quantify key elements for efficacy and safety of MSCs used for periodontal regeneration and (b) to identify patterns in the existing literature to explain differences between studies and suggest recommendations for future research. This systematic review provided good evidence of the capacity of MSCs to regenerate periodontal tissues in animals; however, experimentally generated defects used in animal studies do not sufficiently mimic the pathophysiology of periodontitis in humans. Moreover, the safety of such interventions in humans still needs to be studied. There were marked differences between experimental and control groups that may be influenced by characteristics that are crucial to address before translation to human clinical trials. We suggest that the appropriate combination of cell source, carrier type, and biomolecules, as well as the inclusion of critical path issues for a given clinical case, should be further explored and refined before transitioning to clinical trials. Future studies should investigate periodontal regenerative procedures in animal models, including rodents, in which the defects generated are designed to more accurately reflect the inflammatory status of the host and the shift in their pathogenic microflora. STEM CELLS TRANSLATIONAL MEDICINE *2014;3:1–7*

INTRODUCTION

Periodontitis is a chronic infectious disease of the soft and hard tissues supporting the teeth [1], affecting 15%–50% of adults in developed countries [2]. This disease leads to the formation of deep infrabony defects and soft-tissue crevices called "periodontal pockets" between the tooth and its bony socket. Left untreated, periodontitis can result in tooth loss [1], with a significant impact on oral health and overall quality of life [3, 4]. Because of its infectious and inflammatory sides, periodontitis has been associated with adverse pregnancy outcomes, cardiovascular events, pulmonary disease, or diabetes [5]. Conventional periodontal therapy involves debridement of the root surface to induce healing by repair or regenerative pathways. For decades, several regenerative procedures have been used, including enamel matrix-derived (EMD) proteins [6], guided tissue regeneration [7], and bone graft placement [6].

The treatment of infrabony defects is associated with a high degree of variability in clinical outcomes [8]. Consequently, the regeneration of bone, cementum, and an effective periodontal ligament (PDL) remains a challenge. New treatments for periodontitis have to be developed to better reflect its etiopathophysiology. Recent advances in regenerative medicine have paved the way for such improvements by presenting innovative and imaginative opportunities for periodontal tissue engineering [9]. The main goals of regenerative therapy are to enhance the migration, proliferation, and commitment of endogenous and/or exogenous progenitor/stem cells to appropriate terminally differentiated phenotypes and to favor the biosynthesis of the extracellular matrix components that support tissue recovery [9]. However, genetic determinants, physiological and systemic conditions, and local disease state (infection, scars) are predicted to impair regenerative potential [10, 11]. Grafting exogenous cells to favor the production of new tissues and/or to make the local microenvironment more suitable for stimulation of endogenous progenitors [12, 13] has been tested with promising results. Indeed, successful treatment of various diseases, including cardiovascular diseases and large bone defects treated by cell therapy based on the use of adult multipotent mesenchymal stromal cells (MSCs), has been

reported [14–16]. MSCs have the capacity for mesenchymal lineage plasticity and display sensitivity to local paracrine activity [17]. Because of these unique properties, MSCs delivered in situ to periodontal defects may exert their effects at multiple levels, including neovascularization [18], immunomodulation [19, 20], and tissue regeneration.

Translation of experimental outcomes achieved in animals to human clinical trials requires that the design and results from animal studies should be analyzed with appropriate tools based on rational criteria and strategies. The use of systematic reviews in biology is growing but has never been applied in the field of cell therapy for periodontal regeneration, although some literature reviews devoted to oral tissue regeneration by stem cells have been published previously [9, 21, 22]. A systematic approach is able to minimize the risk of selection bias in the review process. The goals of this systematic review are to objectively quantify key elements for efficacy and safety of MSCs used for periodontal regeneration and to identify patterns in the existing literature to explain differences between studies.

Materials and Methods

This systematic review follows configurative logic that provides insight through an iterative literature search strategy that allows for new ways of understanding existing literature and suggests ways to improve future studies [23]. The methodology used in this review is based on the guidelines for scoping reviews, as suggested by Arksey and O'Malley, and was applied as follows: identify the research questions; search for relevant studies; select appropriate studies; chart the data; collate, summarize, and report the results [24].

Identifying the Research Questions

This systematic review was designed to answer the following questions: What are the different methods used to quantify the effect of MSCs on periodontal regeneration? What is the current evidence on the efficacy and the safety of MSCs used for periodontal regeneration? How can possible differences between studies be explained?

Searching for Relevant Studies

We used a combination of a protocol-driven method and a "snowballing technique" to search for relevant studies. Details of the electronic search strategy in eight databases are provided in the supplemental online data for this study. The search strategy was designed to include data from both animal and human studies. The languages of publication were restricted to English, French, Spanish, and Portuguese. Reference lists of query studies were inspected to identify any additional relevant published or unpublished data. Details of the literature search in the "gray literature" are also provided in the supplemental online data.

Study Selection

Configurative logic was chosen for the selection of relevant studies. By using an iterative process, we determined clear macro rules for inclusion of studies and assumed that specific detailed criteria for meeting those rules would become apparent through the process of doing the review.

All studies dealing with regeneration of deep periodontal tissues (cementum, periodontal ligament, and alveolar bone), with a control group for animal studies, were considered to be eligible. All animal species were considered. With regard to periodontal conditions, there was no restriction regarding the manner in which periodontal lesions were induced. All types of defined MSCs were considered. For outcomes, only studies with quantitative outcomes for both experimental and control groups were selected, and outcomes were listed to answer the research question on the different methods used to quantify the effect of MSCs on periodontal regeneration (supplemental online Fig. 1).

Data Extraction and Subgroup Analyses

We extracted data concerning animal model (gender and species), method used to generate periodontal defects, type of periodontal defect, cell source, type of cell carrier, randomization, study duration, and quality of reporting. We planned to determine whether differences in the efficacy or safety of periodontal regeneration between experimental and control groups were influenced by these characteristics. The chi-square test was used to perform subgroup analyses if at least three groups of two or more studies were available.

Statistical Analysis and Data Charting

Animal and human studies were analyzed separately. We performed meta-analyses on standardized mean differences using an inverse variance random-effects method. Review Manager 5.2 (The Cochrane Collaboration, Copenhagen, Denmark, http://www.cochrane.org) was used to plot the data. Only studies with an appropriate control group were considered for quantitative analysis. When a study had several experimental groups, each group was included separately in the quantitative analysis. The funnel plot was visually examined, and an Egger's test was performed using the "metabias" command in Stata 11.1 (StataCorp, College Station, TX, http://www.stata.com) to determine publication bias. The studies determined to be significantly responsible for publication bias (e.g., increasing heterogeneity) were removed from the meta-analysis and discussed separately.

Results

Seventy publications from 2,986 records met the inclusion criteria and were included in the descriptive synthesis (supplemental online Fig. 2 and supplemental online Table 1 show details of the selection process). Finally, 56 studies (45 animal studies, 11 human studies) were included.

Periodontal Regeneration in Animal Studies

Characteristics of each study are shown in supplemental online Table 3 and summarized in supplemental online Table 2.

Description of Included Studies

We found comparable numbers of studies on periodontal regeneration of fenestration defects, infrabony defects, and furcation defects. To induce a periodontal defect, periodontal bone was removed with surgical burs (74% of total selected articles) or induced by creating an additional deep inflammatory process within the periodontium using impression paste, gutta-percha, or a ligature around the cervical part of the tooth (21%). Experiments were conducted on dogs in 49%, pigs in 14% and rodents in 33% of included studies. Cells were derived from oral tissues in

63% of studies, and embryonic stem cells and induced pluripotent stem cells were used in four studies. Engraftment was autologous in 63%, allogeneic in 14%, and xenogeneic in 28% of included studies. Cells were carried within cell sheets in 23% of studies. Extracellular matrix proteins (collagen, fibrin, gelatin, or hyaluronic acid) were used in 49% of included studies, enamel matrix derivatives were used in 5%, plasma-rich platelet (PRP) concentrate or blood coagulum was used in 9%, and polymers (alginate, poly-ε-caprolactone, polyglycolic acid, silk fibroin, or pluronic F127) were used in 23%. A guided tissue regeneration technique [7] was used in 30% of included studies. Quality of periodontal regeneration cell therapy reported for animal studies was investigated and summarized in supplemental online Figure 3.

Methods Used to Quantify Periodontal Regeneration

A variety of methods were used by the authors to quantify periodontal regeneration. Histological techniques (cementum, bone regeneration) were reported in 91%, radiological measures (bone volume) were reported in 14%, biological analyses (percentage of cells, fluorescence intensity) were reported in 5%, clinical assessment (clinical attachment level [CAL], periodontal pocket depth [PPD]) was reported in 37%, and safety appreciation (postoperative complications, neoplasm formation) was reported in 63% of studies.

Efficacy of Periodontal Regeneration

Bone and cementum regeneration were the most frequently reported outcomes. Bone regeneration was investigated by microscopic and/or radiography examination in 28 studies. Funnel plot and Egger's test (supplemental online Fig. 4A) suggested publication bias ($p < .05$) that could be attributed to four studies [25–28]; therefore, these were removed from analysis. The forest plot performed on the remaining 24 studies showed that alveolar bone regeneration was significantly enhanced by MSC therapy (mean difference: 0.66 [95% confidence interval (CI): 0.37–0.95]) compared with control groups treated without MSCs (Fig. 1). Cementum regeneration was investigated by microscopic examination in 18 studies. One study [27] had to be removed from analysis (Egger's test, $p < .05$) (supplemental online Fig. 4B). Once removed, the forest plot performed on the remaining 17 studies (Fig. 2) showed that cementum regeneration was significantly promoted by MSC therapy (mean difference: 0.93 [95% CI: 0.62–1.25]).

Safety Outcomes

There were 19 studies that reported postoperative complications. In one study [54], wound dehiscence was observed twice in four animals from the experimental group. Nine studies reported bone ankylosis, although no difference was observed between intervention and control groups. Eight studies reported root resorption; in seven of these, the number of samples with resorption was the same in the experimental and control groups. One study [55] showed resorption events for the control group only. No formation of a neoplasm occurred in the four studies that investigated this kind of adverse effect. Nevertheless, we identified two conference presentations on neoplasm formation after implantation of human periodontium-derived stem cells in periodontal defects in rats. One of the presentations [56] reported observing anaplastic squamous cell carcinoma in 6 of 11 rats. Another study [57] reported tumor initiation in 50% of immune-deficient rats.

Subgroup Analyses

In order to investigate whether outcomes—more precisely, the magnitude of the difference between experimental and control groups—could have been influenced by study characteristics, we performed several subgroup analyses. We found that bone and cementum regeneration was statistically greater in the experimental group (delivered cells) compared with controls when cells were grafted with bone substitute (mean difference: 0.81 [95% CI: 0.22–1.39] and 0.92 [95% CI: 0.35–1.49]) or with collagen, fibrin, hydrogel, gelatin, or hyaluronic acid carriers (mean difference: 0.79 [95% CI: 0.43–1.14] and 1.12 [95% CI: 0.65, 1.59]) than with PRP, EMD, blood clots, cell sheets, or polymers. Autologous grafts (mean difference: 0.71 [95% CI: 0.45–0.97]) and allogeneic grafts (mean difference: 1.86 [95% CI: 0.73–2.98]) enhanced significantly more bone regeneration than xenogeneic grafting (mean difference: 0.07 [95% CI: −0.77 to 0.91]) ($p = .04$). Whatever the cell source, experimental groups displayed statistically greater bone and cementum regeneration compared with control. Cementum regeneration by bone marrow stromal cells (BMSCs) or adipose-derived stromal cells (ADSCs) (mean difference: 1.03 [95% CI: −0.16 to 2.23]) was nearly significant. More details are provided in supplemental online Table 4A and 4B.

Periodontal Regeneration in Human Studies

We identified seven human case reports (supplemental online Table 5) in which periodontal regeneration was achieved. Studies investigated periodontal ligament stromal cells (two studies), BMSCs (one study), and cells derived from gingiva (three studies) or periosteum (two studies). Among the three studies using a control group, two reported data from MSCs in combination with hydroxyapatite (HA) versus HA alone, suggesting higher probing-depth reduction [58] and attachment gain [58, 59] in defects treated with gingival or periodontal ligament cells compared with controls. Improvement of CAL and PPD in the group treated with BMSCs compared with controls was also reported [60]. In addition, we identified four clinical trials from our search of the gray literature (supplemental online Table 5).

DISCUSSION

This systematic review provides evidence of the capacity of MSCs to regenerate periodontal tissues in animals; however, the safety of such interventions in humans still needs to be investigated. Subgroup analyses showed marked differences between experimental and control groups, suggesting that periodontal regeneration may be influenced by variables (e.g., carriers or cells sources) that are crucial to address before translation to human clinical trials.

The efficacy of periodontal regeneration techniques has been predominantly assessed using a quantitative approach (amount of tissues), but now investigation with greater statistical rigor is required to study outcomes related to the inherent structural aspects of the formation of a new functional attachment, the bone quality (e.g., cellularity), the type of cementum (i.e., regeneration of acellular extrinsic fiber cementum), and the hierarchy and organization of the periodontal ligament fibers [61].

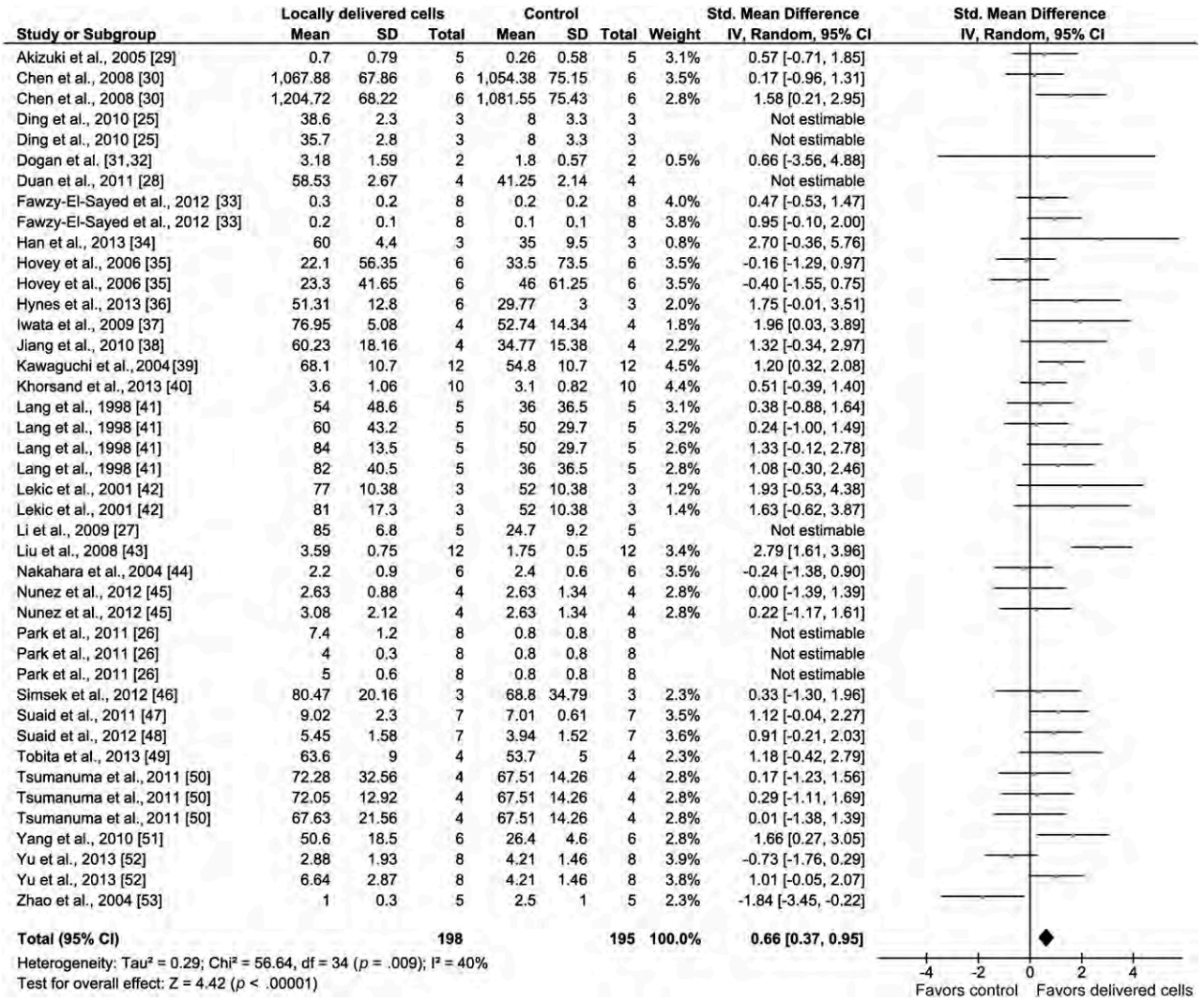


Figure 1. Forest plot representing the 24 studies on alveolar bone regeneration by cell therapy. The result of each individual study with its confidence interval was plotted, and then a weighted average was calculated. The pooled analysis was given a diamond shape; the widest aspect of this shape is located at the global estimate and the corresponding horizontal width is the confidence interval. A random effect meta-analysis with the inverse variance method was used to obtain the global standardized mean difference with a 95% confidence interval. Bone regeneration was significantly higher in the cell therapy group than in the control group. Abbreviations: CI, confidence interval; df, degrees of freedom; IV, inverse variance; Std., standard.

The question of which biomaterial to use to support cell delivery is critical in treating mineralized tissue defects because it is well known that the outcomes of surgical therapy on periodontal pockets may depend on the scaffold used [62]. Carriers should mimic the cell microenvironment, in which extracellular substrates are able to contribute to their control over cell fate acquisition [63] while permitting cell-to-cell and cell-to-matrix interactions [64, 65]. Interestingly, this review did not find evidence of significant bone or cementum regeneration when cells were delivered as cell sheets or with EMD or PRP adjunctive therapy when compared with carrier alone. Because EMD and PRP are already used when treating humans with the intent of periodontal regeneration, and because in vitro studies previously showed that these materials or delivery systems promoted MSC differentiation [66], their lack of efficiency in cell therapy for periodontal pockets is surprising. Consequently, we hypothesized that cell sheets, EMD, and PRP may not be suitable as carriers in the type of defects created in these particular studies, which were mainly furcation defects in dogs. We showed that bone substitutes enhanced the efficiency of grafted MSCs in experimental periodontal defects probably because they fill the wound, stabilize the blood clot, and confine the cells within the surgical site without rapid resorption, which could alter the local environment for regeneration [67]. For now, given the biomaterials in our possession for safe and routine use, we suggest that the best way of delivering cells may depend on the type of defect and, specifically, the number of alveolar bone walls involved. When lesions are retentive, liquid or gel scaffolds might be used without risking cell dispersal. When defects are larger, outcomes may improve when cells are associated with bone substitute that confines them to the surgical site. Our analysis pointed out that it is not currently possible to state whether the use of MSCs for periodontal regeneration gives better results than conventional therapies. We suggest that future animal studies should compare cell therapy with

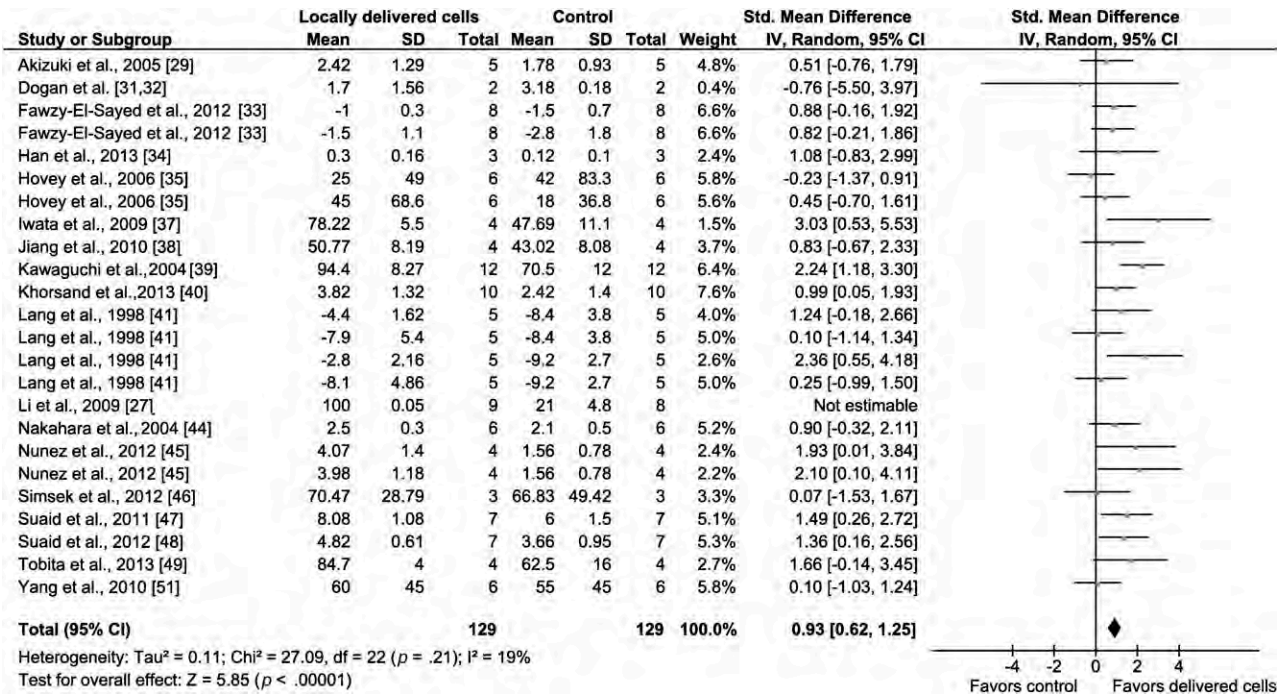


Figure 2. Forest plot representing the 17 studies on cementum regeneration by cell therapy. A random effect meta-analysis with the inverse variance method was used to obtain standardized mean difference with a 95% confidence interval. Cementum regeneration was significantly higher in the cell therapy group than in the control group. Abbreviations: CI, confidence interval; df, degrees of freedom; IV, inverse variance; Std., standard.

current conventional periodontal regenerative therapies, with special focus on the type of cell-supporting scaffolds, and should use a split-mouth strategy to reduce interindividual variability.

Another key issue is the availability of cells. Given that periodontitis is not a terminal disease, the risk-benefit balance requires that any therapy should be strongly justified. Thus, sampling should be easy and painless and have a low risk of complications. Our systematic review showed that periodontal therapy with xenogeneic stem cells did not bring about positive outcomes. The only way to bypass the lack of availability of MSCs at the time of treatment appears to be to use frozen autologous or allogeneic cells. Five studies suggested that cryopreservation did not alter periodontal regenerative potential of MSCs. Nevertheless, except for one study [27], no adequate control group was present (e.g., a cell group without cryopreservation) to draw any robust conclusion. Many studies have examined the influence of the source of MSCs on their capacity to participate in periodontal regeneration. Obviously, oral cells appeared to be good candidates for periodontal therapy; however, because these cells are derived from PDL, dental pulp, gingiva, alveolar bone, or dental follicle cells, they are not always conveniently available in clinical practice. It is crucial to have other nonoral MSC sources. Data analyzed in this review showed that cells from extraoral bone marrow sites or from adipose tissue were comparable to adult mesenchymal stem cells derived from dental tissues in their ability to regenerate periodontal bone. This result confirmed that the microenvironment and surrounding tissue were important factors that influenced the fate of MSCs ultimately used [63]. Indeed, BMSCs were shown to shift toward periodontal ligament-like cell features when cocultured with the periodontal ligament in vivo [68]. In addition, because they have been used successfully in extraoral connective tissue regeneration [69], adipose-derived stromal cells are expected to be a valuable source of cells. These ADSCs can be easily isolated from intact resected adipose tissue or by using liposuction, and their properties are similar to cells isolated from bone marrow [14, 15]. However, quantitative data regarding the capacity of ADSCs to contribute to periodontal regeneration were sparse at the time of writing [70, 71].

Interpretation of data from this systematic review should be made with caution and balanced according to the limitations of reported data contained in studies included in this review. We suggest that methodological aspects of animal experiments should be a focus for improvement. It is important to calculate the number of animals that should be treated to obtain a power analysis predictive of reliable results, yet such calculations were reported in only one study [55]. Randomization of the defects to be attributed to treatment groups was performed in only 49% of studies. Six studies lacked control groups or did not compare regeneration with MSCs with a similar experimental design without MSCs. Methods used to minimize subjective bias, the reporting of animal randomization, and blinding design should also be a focus for improvement, and with these improvements, the selection and detection bias should also be minimized [72]. Finally, experimentally generated defects used in most of the animal studies do not mimic the natural pathophysiology of periodontitis, and the method of lesion induction might bias therapeutic outcomes. For future studies, we suggest that periodontal regenerative procedures should be investigated in animal models, including rodents, in which the defects that are generated more accurately reflect both the inflammatory status and the pathogenic microflora shift seen in periodontitis [73]. Periodontal cell therapy should also be explored in large animals. In particular, the minipig model seems

to be relevant because the morphology of teeth and anatomy of the periodontal region in swine are close phenocopies for human traits; moreover, in the minipigs, periodontitis occurs spontaneously from 16 months of age [25, 55].

Conclusion

Given the heterogeneity of present studies, narrative reviews are insufficient. A systematic approach with meta-analysis is essential to provide guidance to support future studies and should provide data that can be generalized. The challenge remains to identify the best combination of cells, biomaterials, and biomolecules for various clinical situations, using animal models that best represent the etiopathophysiology of human periodontitis. Particular attention should be given to methodology used in randomized clinical trials. Moreover, even if some clinical trials are already recruiting, animal research should be maintained in parallel.

Acknowledgments

This study was supported by funding from the Université de Toulouse, the Toulouse Faculty of Dentistry, the Paul Sabatier University, and the Toulouse University Hospital (P.M., J.-N.V., C.N., V.P.B., L.C., P.K.). M.L.S. was supported by a grant from the U.S. Public Health Service, National Institutes of Health, National Institute of Dental and Craniofacial Research (DE13045). We thank Bridget Samuels (University of Southern California) for proofreading the manuscript.

Author Contributions

P.M.: conception and design, collection and/or assembly of data, data analysis and interpretation, manuscript writing; J.-N.V.: conception and design, data analysis and interpretation, manuscript writing; C.N.: conception and design, final approval of manuscript; M.S.: administrative support, financial support, final approval of manuscript; M.L.S.: data analysis and interpretation, manuscript writing, final approval of manuscript, manuscript editing; V.P.-B.: conception and design, manuscript writing, final approval of manuscript; L.C.: conception and design, financial support, manuscript writing, final approval of manuscript; P.K.: administrative support, collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript.

Disclosure of Potential Conflicts of Interest

The authors indicate no potential conflicts of interest.

References

1 Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. Lancet 2005;366:1809–1820.
2 Albandar JM. Periodontal disease surveillance. J Periodontol 2007;78:1179–1181.
3 Gerritsen AE, Allen PF, Witter DJ et al. Tooth loss and oral health-related quality of life: A systematic review and meta-analysis. Health Qual Life Outcomes 2010;8:126.
4 Williams RC, Barnett AH, Claffey N et al. The potential impact of periodontal disease on general health: A consensus view. Curr Med Res Opin 2008;24:1635–1643.
5 Cullinan MP, Seymour GJ. Periodontal disease and systemic illness: Will the evidence ever be enough? Periodontol 2000 2013;62:271–286.
6 Esposito M, Grusovin MG, Papanikolaou N et al. Enamel matrix derivative (Emdogain(R)) for periodontal tissue regeneration in intrabony defects. Cochrane Database Syst Rev 2009;CD003875.
7 Needleman IG, Worthington HV, Giedrys-Leeper E et al. Guided tissue regeneration for periodontal infra-bony defects. Cochrane Database Syst Rev 2006;CD001724.
8 Aichelmann-Reidy ME, Reynolds MA. Predictability of clinical outcomes following regenerative therapy in intrabony defects. J Periodontol 2008;79:387–393.
9 Hynes K, Menicanin D, Gronthos S et al. Clinical utility of stem cells for periodontal regeneration. Periodontol 2000 2012;59:203–227.
10 Rao MS, Mattson MP. Stem cells and aging: Expanding the possibilities. Mech Ageing Dev 2001;122:713–734.
11 Yue WS, Lau KK, Siu CW et al. Impact of glycemic control on circulating endothelial progenitor cells and arterial stiffness in patients with type 2 diabetes mellitus. Cardiovasc Diabetol 2011;10:113.
12 Ilic D, Polak J. Stem cell based therapy—where are we going? Lancet 2012;379:877–878.
13 Shin L, Peterson DA. Human mesenchymal stem cell grafts enhance normal and impaired wound healing by recruiting existing endogenous tissue stem/progenitor cells. Stem Cells Translational Medicine 2013;2:33–42.
14 Bourin P, Sensebé L, Planat-Bénard V et al. Culture and use of mesenchymal stromal cells in phase I and II clinical trials. Stem Cells Int 2010;503593.
15 Bourin P, Bunnell BA, Casteilla L et al. Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: A joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). Cytotherapy 2013;15:641–648.
16 Dominici M, Le Blanc K, Mueller I et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006;8:315–317.
17 Psaltis PJ, Zannettino AC, Worthley SG et al. Concise review: Mesenchymal stromal cells: Potential for cardiovascular repair. Stem Cells 2008;26:2201–2210.
18 Wu Y, Chen L, Scott PG et al. Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. Stem Cells 2007;25:2648–2659.
19 Koch CA, Geraldes P, Platt JL. Immunosuppression by embryonic stem cells. Stem Cells 2008;26:89–98.
20 Shi Y, Hu G, Su J et al. Mesenchymal stem cells: A new strategy for immunosuppression and tissue repair. Cell Res 2010;20:510–518.
21 Bartold PM, Shi S, Gronthos S. Stem cells and periodontal regeneration. Periodontol 2000 2006;40:164–172.
22 Bartold PM, Xiao Y, Lyngstaadas SP et al. Principles and applications of cell delivery systems for periodontal regeneration. Periodontol 2000 2006;41:123–135.
23 Gough D, Thomas J, Oliver S. Clarifying differences between review designs and methods. Syst Rev 2012;1:28.
24 Arksey H, O'Malley L. Scoping studies: Towards a methodological framework. Int J Soc Res Methodol 2005;8:19–32.
25 Ding G, Liu Y, Wang W et al. Allogeneic periodontal ligament stem cell therapy for periodontitis in swine. Stem Cells 2010;28:1829–1838.
26 Park JY, Jeon SH, Choung PH. Efficacy of periodontal stem cell transplantation in the treatment of advanced periodontitis. Cell Transplant 2011;20:271–285.
27 Li H, Yan F, Lei L et al. Application of autologous cryopreserved bone marrow mesenchymal stem cells for periodontal regeneration in dogs. Cells Tissues Organs 2009;190:94–101.
28 Duan X, Tu Q, Zhang J et al. Application of induced pluripotent stem (iPS) cells in periodontal tissue regeneration. J Cell Physiol 2011;226:150–157.
29 Akizuki T, Oda S, Komaki M et al. Application of periodontal ligament cell sheet for periodontal regeneration: A pilot study in beagle dogs. J Periodontal Res 2005;40:245–251.
30 Chen YL, Chen PK, Jeng LB et al. Periodontal regeneration using ex vivo autologous stem cells engineered to express the BMP-2 gene: An alternative to alveolaplasty. Gene Ther 2008;15:1469–1477.
31 Dogan A, Ozdemir A, Kubar A et al. Assessment of periodontal healing by seeding of fibroblast-like cells derived from regenerated periodontal ligament in artificial furcation defects in a dog: A pilot study. Tissue Eng 2002;8:273–282.
32 Dogan A, Ozdemir A, Kubar A et al. Healing of artificial fenestration defects by seeding of fibroblast-like cells derived from regenerated

periodontal ligament in a dog: A preliminary study. Tissue Eng 2003;9:1189–1196.

33 Fawzy El-Sayed KM, Paris S, Becker ST et al. Periodontal regeneration employing gingival margin-derived stem/progenitor cells: An animal study. J Clin Periodontol 2012;39: 861–870.

34 Han J, Menicanin D, Marino V et al. Assessment of the regenerative potential of allogeneic periodontal ligament stem cells in a rodent periodontal defect model. J Periodontal Res 2013 [Epub ahead of print].

35 Hovey LR, Jones AA, McGuire M et al. Application of periodontal tissue engineering using enamel matrix derivative and a human fibroblast-derived dermal substitute to stimulate periodontal wound healing in Class III furcation defects. J Periodontol 2006;77:790–799.

36 Hynes K, Menicanin D, Han J et al. Mesenchymal stem cells from iPS cells facilitate periodontal regeneration. J Dent Res 2013;92: 833–839.

37 Iwata T, Yamato M, Tsuchioka H et al. Periodontal regeneration with multi-layered periodontal ligament-derived cell sheets in a canine model. Biomaterials 2009;30:2716–2723.

38 Jiang J, Wu X, Lin M et al. Application of autologous periosteal cells for the regeneration of class III furcation defects in Beagle dogs. Cytotechnology 2010;62:235–243.

39 Kawaguchi H, Hirachi A, Hasegawa N et al. Enhancement of periodontal tissue regeneration by transplantation of bone marrow mesenchymal stem cells. J Periodontol 2004; 75:1281–1287.

40 Khorsand A, Eslaminejad MB, Arabsolghar M et al. Autologous dental pulp stem cells in regeneration of defect created in canine periodontal tissue. J Oral Implantol 2013;39: 433–443.

41 Lang H, Schuler N, Nolden R. Attachment formation following replantation of cultured cells into periodontal defects—a study in minipigs. J Dent Res 1998;77:393–405.

42 Lekic PC, Rajshankar D, Chen H et al. Transplantation of labeled periodontal ligament cells promotes regeneration of alveolar bone. Anat Rec 2001;262:193–202.

43 Liu Y, Zheng Y, Ding G et al. Periodontal ligament stem cell-mediated treatment for periodontitis in miniature swine. STEM CELLS 2008;26:1065–1073.

44 Nakahara T, Nakamura T, Kobayashi E et al. In situ tissue engineering of periodontal tissues by seeding with periodontal ligament-derived cells. Tissue Eng 2004;10:537–544.

45 Nunez J, Sanz-Blasco S, Vignoletti F et al. Periodontal regeneration following implantation of cementum and periodontal ligament-derived cells. J Periodontal Res 2012;47:33–44.

46 Simsek SB, Keles GC, Baris S et al. Comparison of mesenchymal stem cells and autogenous cortical bone graft in the treatment of class II furcation defects in dogs. Clin Oral Investig 2012;16:251–258.

47 Suaid FF, Ribeiro FV, Rodrigues TL et al. Autologous periodontal ligament cells in the treatment of class II furcation defects: A study in dogs. J Clin Periodontol 2011;38:491–498.

48 Suaid FF, Ribeiro FV, Gomes TR et al. Autologous periodontal ligament cells in the treatment of Class III furcation defects: A study in dogs. J Clin Periodontol 2012;39:377–384.

49 Tobita M, Uysal CA, Guo X et al. Periodontal tissue regeneration by combined implantation of adipose tissue-derived stem cells and platelet-rich plasma in a canine model. Cytotherapy 2013;15:1517–1526.

50 Tsumanuma Y, Iwata T, Washio K et al. Comparison of different tissue-derived stem cell sheets for periodontal regeneration in a canine 1-wall defect model. Biomaterials 2011;32: 5819–5825.

51 Yang Y, Rossi FM, Putnins EE. Periodontal regeneration using engineered bone marrow mesenchymal stromal cells. Biomaterials 2010; 31:8574–8582.

52 Yu N, Oortgiesen DA, Bronckers AL et al. Enhanced periodontal tissue regeneration by periodontal cell implantation. J Clin Periodontol 2013;40:698–706.

53 Zhao M, Jin Q, Berry JE et al. Cementoblast delivery for periodontal tissue engineering. J Periodontol 2004;75:154–161.

54 Wei N, Gong P, Liao D et al. Autotransplanted mesenchymal stromal cell fate in periodontal tissue of beagle dogs. Cytotherapy 2010;12:514–521.

55 Fawzy El-Sayed KM, Paris S, Becker ST et al. Periodontal regeneration employing gingival margin-derived stem/progenitor cells: An animal study. J Clin Periodontol 2012;39: 861–870.

56 Dannan A, Becher S, Dittmar T et al. Human periodontium-derived stem cells (pdSCs) may give to tumorigenesis. Presented at: EuroPerio 6 conference; June 4–6, 2009; Stockholm, Sweden.

57 Grimm W, Becher S, Dannan A et al. Evaluation of an in vivo animal model for the transplantation of human periodontium-derived stem cells (pdSCs). Presented at: EuroPerio 5 conference; June 29–July 1, 2006; Madrid, Spain.

58 Hou LT, Tsai AY, Liu CM et al. Autologous transplantation of gingival fibroblast-like cells and a hydroxylapatite complex graft in the treatment of periodontal osseous defects: Cell cultivation and long-term report of cases. Cell Transplant 2003;12:787–797.

59 Feng F, Hou LT. Treatment of osseous defects with fibroblast-coated hydroxylapatite particles. J Formos Med Assoc 1992;91:1068–1074.

60 Yamada Y, Ueda M, Hibi H et al. A novel approach to periodontal tissue regeneration with mesenchymal stem cells and platelet-rich plasma using tissue engineering technology: A clinical case report. Int J Periodontics Restorative Dent 2006;26:363–369.

61 Bosshardt DD, Selvig KA. Dental cementum: The dynamic tissue covering of the root. Periodontol 2000 1997;13:41–75.

62 Kolind K, Leong KW, Besenbacher F et al. Guidance of stem cell fate on 2D patterned surfaces. Biomaterials 2012;33:6626–6633.

63 Chen FM, Wu LA, Zhang M et al. Homing of endogenous stem/progenitor cells for in situ tissue regeneration: Promises, strategies, and translational perspectives. Biomaterials 2011; 32:3189–3209.

64 Chen FM, Zhang J, Zhang M et al. A review on endogenous regenerative technology in periodontal regenerative medicine. Biomaterials 2010;31:7892–7927.

65 Chen FM, Sun HH, Lu H et al. Stem cell-delivery therapeutics for periodontal tissue regeneration. Biomaterials 2012;33:6320–6344.

66 Kémoun P, Gronthos S, Snead ML et al. The role of cell surface markers and enamel matrix derivatives on human periodontal ligament mesenchymal progenitor responses in vitro. Biomaterials 2011;32:7375–7388.

67 Karageorgiou V, Kaplan D. Porosity of 3D biomaterial scaffolds and osteogenesis. Biomaterials 2005;26:5474–5491.

68 Kramer PR, Nares S, Kramer SF et al. Mesenchymal stem cells acquire characteristics of cells in the periodontal ligament in vitro. J Dent Res 2004;83:27–34.

69 Casteilla L, Planat-Bénard V, Dehez S et al. Endothelial and cardiac regeneration from adipose tissues. Methods Mol Biol 2011;702: 269–287.

70 Tobita M, Uysal CA, Guo X et al. Periodontal tissue regeneration by combined implantation of adipose tissue-derived stem cells and platelet-rich plasma in a canine model. Cytotherapy 2013;15:1517–1526.

71 Takedachi M, Sawada K, Yamamoto S et al. Periodontal tissue regeneration by transplantation of adipose tissue-derived stem cells. J Oral Biosci 2013;55:137–142.

72 Higgins JPT, Green S, eds. , eds. Cochrane Handbook for Systematic Reviews of Interventions, version 5.1.0 (updated March 2011). London, U.K.: The Cochrane Collaboration, 2011.

73 Graves DT, Fine D, Teng YT et al. The use of rodent models to investigate host-bacteria interactions related to periodontal diseases. J Clin Periodontol 2008;35:89–105.

See www.StemCellsTM.com for supporting information available online.

Supplementary Material Supplementary material can be found at:
http://stemcellstm.alphamedpress.org/content/suppl/2014/04/17/sctm.2013-0183.DC1.html

Searching for relevant studies

Described below are the search strategies used for each queried database (see "Materials and Methods").

The last search was conducted on 2013/08/25.
We searched the following databases: Cochrane Central Register of Controlled Trials; Health Technologies Assessment (HTA); Database of Abstracts of Reviews of Effects (DARE); Ovid MEDLINE In-Process and Other Non-Indexed Citations via OvidSP; the databases of MEDLINE (1950 to present) via Pubmed; Biosis (1997 to present) via OvidSP®; PASCAL (1984 to present) via EBSCOHost and LILACS via the Virtual Health Library search form (1982 to present).

Reference lists of query studies were inspected to identify any additional relevant published or unpublished data. We did not contact the authors of the studies. For literature that is difficult to trace, the so-called "grey literature," we sought out unpublished and on-going trials by searching the World Health Organization (WHO) International Clinical Trials Registry Platform [1], which provides access to several trial registries including ClinicalTrials.gov and the International Standard Randomized Controlled Trial Number Register (ISRCTN). We also searched for Conference proceedings and data from two major meetings: the EUROPERIO meeting [2] sessions from 1997 to present, and International Association for Dental Research meeting [3] (IADR) sessions from 2001 to present.

MEDLINE via Pubmed

- #1: Periodontal Diseases [mh]
- #2: Alveolar Bone Loss [mh:noexp]
- #3: Periodontics [mh]
- #4: periodont* [tw] OR parodont* [tw]
- #5: ((gum[tw] OR alveolar bone [tw]) AND (loss* [tw] OR atroph* [tw] OR disease [tw] OR diseases [tw]))
- #6: Stem Cell Transplantation [mh]
- #7: Stem Cells [mh]
- #8: Bone Marrow Transplantation [mh:noexp]
- #9: Hematopoietic Stem Cells [mh]
- #10: Cell Transplantation [mh:noexp]
- #11: (cell[tw] OR cells [tw]) AND (transplant* [tw] OR graft* [tw] OR implant* [tw])
- #12: multipotent[tw] OR pluripotent [tw] OR totipotent [tw] OR stem [tw] OR progenitor OR precursor OR (mesenchymal AND stromal)

- #13: #1 OR #2 OR #3 OR #4 OR #5
- #14: #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12
- #15: #13 AND #14

PASCAL via EBSCOHost

- S1: TX periodont* OR TX parodont*
- S2: TX ((gum* OR alveolar bone) N2 (loss* OR atroph* OR diseas*))
- S3: TX (cell* N3 (transplant* OR graft* OR implant*))
- S4: TX (multipotent N2 cell* OR pluripotent N2 cell* OR totipotent N2 cell* OR stem cell* OR progenitor N2 cell* OR precursor N2 cell* OR cell* therap* OR (mesenchymal N1 stromal))
- S5: S1 OR S2
- S6: S3 OR S4
- S7: S5 AND S6

CENTRAL via OvidSP

- 1: exp Periodontal Diseases/
- 2: Alveolar Bone Loss/
- 3: exp Periodontics/
- 4: (periodont* or parodont*).tw.
- 5: (alveolar bone or gum*).tw.
- 6: (loss* or atroph* or diseas*).tw.
- 7: 5 adj2 6
- 8: exp Stem Cell Transplantation/
- 9: exp Stem Cells/
- 10: Bone Marrow Transplantation/
- 11: exp Hematopoietic Stem Cells/
- 12: Cell Transplantation/
- 13: (transplant* or graft* or implant*).tw.
- 14: cell*.tw.
- 15: 13 adj3 14
- 16: (multipotent adj2 cell*).tw.
- 17: (pluripotent adj2 cell*).tw.
- 18: (totipotent adj2 cell*).tw.
- 19: (progenitor adj2 cell*).tw.
- 20: (precursor adj2 cell*).tw.

- 21: (stem cell* or cell* therap*).tw.
- 22: (mesenchymal adj1 stromal).tw.
- 23: 16 or 17 or 18 or 19 or 20 or 21 or 22
- 24: 1 or 2 or 3 or 4 or 7
- 25: 8 or 9 or 10 or 11 or 12 or 15 or 23
- 26: 24 and 25

HTA via OvidSP

- 1: exp Periodontal Diseases/ or alveolar bone loss/
- 2: (periodont* or parodont*).tw.
- 3: (alveolar bone or gum*).tw.
- 4: (loss* or atroph* or diseas*).tw.
- 5: 3 adj2 4
- 6: exp Stem Cell Transplantation/
- 7: exp Stem Cells/
- 8: Bone Marrow Transplantation/
- 9: exp Hematopoietic Stem Cells/
- 10: Cell Transplantation/
- 11: (transplant* or graft* or implant*).tw.
- 12: cell*.tw.
- 13: 11 adj3 12
- 14: (multipotent adj2 cell*).tw.
- 15: (pluripotent adj2 cell*).tw.
- 16: (totipotent adj2 cell*).tw.
- 17: (progenitor adj2 cell*).tw.
- 18: (precursor adj2 cell*).tw.
- 19: (stem cell* or cell* therap*).tw.
- 20: (mesenchymal adj1 stromal).tw.
- 21: 14 or 15 or 16 or 17 or 18 or 19 or 20
- 22: 1 or 2 or 5
- 23: 6 or 7 or 8 or 9 or 10 or 13 or 21
- 24: 22 and 23

DARE, Biosis, Ovid MEDLINE® In-Process and Other Non-Indexed Citations via OvidSP®

- 1: (periodont* or parodont*).tw.
- 2: (alveolar bone or gum*).tw.

- 3: (loss* or atroph* or diseas*).tw.
- 4: 2 adj2 3
- 5: (transplant* or graft* or implant*).tw.
- 6: cell*.tw.
- 7: 5 adj3 6
- 8: (multipotent adj2 cell*).tw.
- 9: (pluripotent adj2 cell*).tw.
- 10: (totipotent adj2 cell*).tw.
- 11: (progenitor adj2 cell*).tw.
- 12: (precursor adj2 cell*).tw.
- 13: (stem cell* or cell* therap*).tw.
- 14: (mesenchymal adj1 stromal).tw.
- 15: 8 or 9 or 10 or 11 or 12 or 13 or 14
- 16: 1 or 4
- 17: 7 or 15
- 18: 16 and 17

LILACS

(periodont$ OR parodont$ OR "PERIODONTAL DISEASES" OR "ALVEOLAR BONE LOSS/" OR "PERIODONTICS") AND ("STEM CELL TRANSPLANTATION" OR "STEM CELLS" OR "BONE MARROW TRANSPLANTATION/" OR "HEMATOPOIETIC STEM CELLS" OR "CELL TRANSPLANTATION/" OR multipotent OR pluripotent OR totipotent OR stem OR progenitor OR precursor OR mesenchymal OR stromal) [Words]

International Clinical Trials Registry Platform

periodont* AND stem OR periodont* AND stromal OR periodont* AND multipotent OR periodont* AND pluripotent OR periodont* AND totipotent OR periodont* AND stem cell* OR periodont* AND progenitor cell* OR periodont* AND precursor cell* OR periodont* AND cell* therap* OR periodont* AND mesenchymal

Figure S1: Suggested outcomes for investigating periodontal regeneration. The choice of these markers was inspired by a bio-psychosocial model, and during the review process, we looked to identify these parameters [4].

Figure S1

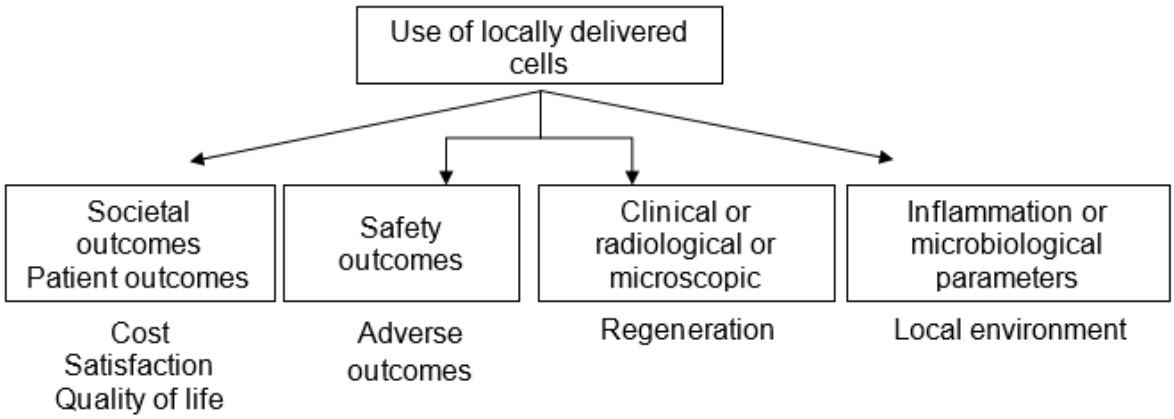


Figure S2: Flow diagram adapted from PRISMA guidelines.

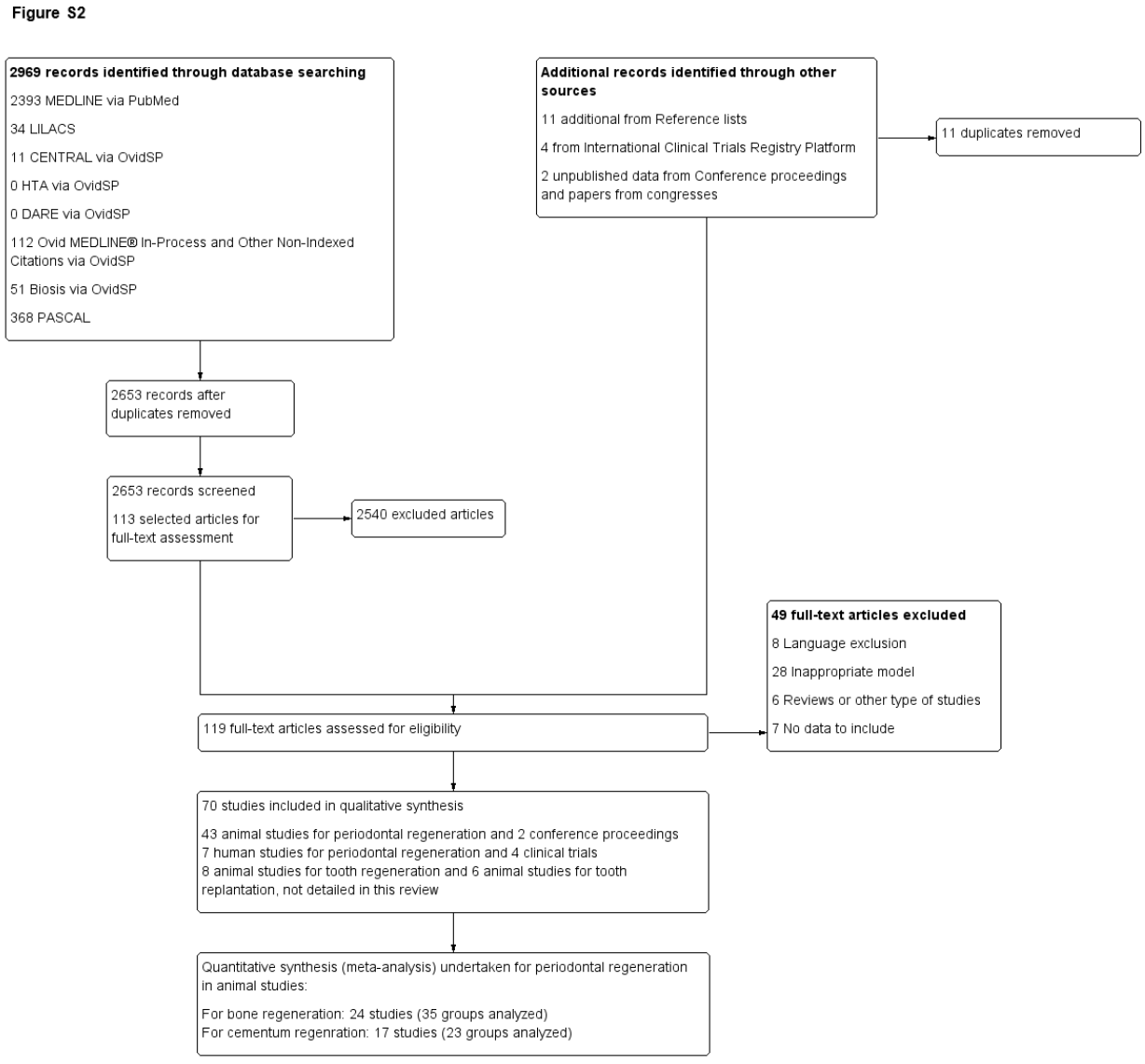
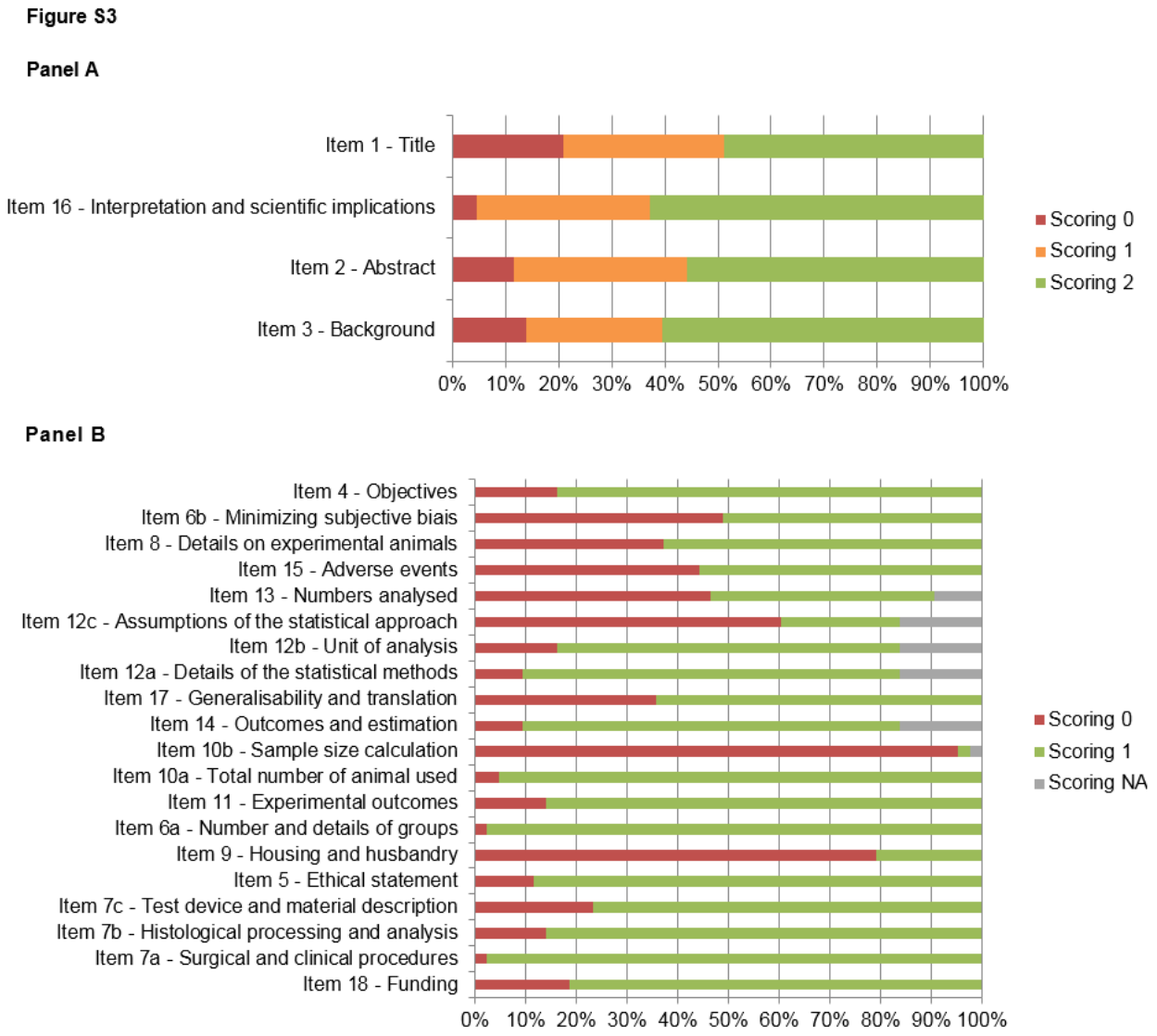


Figure S3: Quality of reporting of included studies.



The quality of reporting of included studies was investigated using a modified version of the ARRIVE (Animal Research: Reporting In Vivo Experiments) guidelines [5, 6]. It comprises an 18-points checklist of the essential information that should be reported. Each item was coded according to standard instructions (0, 1, 2 or not applicable). Studies were classified according to the quality of their reporting: high quality, fourth quartile; moderate quality, third quartile; lower quality, first or second quartile. Assessment of quality of reporting was performed twice by the same author (PM) at an interval of 1 month between assessments.

Frequency distribution (%) of the scores for each parameter of the modified ARRIVE guidelines. (A) Items 1-3 and 16 were coded 0, 1 or 2 (poor, adequate or good) (B) whereas, other items were coded 0, 1 (no or yes) or not applicable (NA).

Overall mean quality was 67.4%±14.1. Crucial methodological and statistical items were lacking in some studies. Indeed, methods to minimize subjective bias when allocating animals or defects to treatment groups or when assessing results were not used in half of the studies; moreover, a priori sample size calculation was not reported, except in one study [7]. Methods used to assess whether the data met the assumptions of the statistical approach were not reported in 60% of studies. The number of analyzed animals and the number of adverse events were not reported in about 45% of studies. Some 50% of the selected articles provided title elements that contributed to an accurate and concise description of the content of the article.

Figure S4: Funnel plots were created by plotting standard errors of intervention effect estimates versus intervention effect estimates **(A)** Funnel plot of bone regeneration with an obvious asymmetry linked to a publication bias (Egger's test < 0.05) attributed to 7 points corresponding to 4 studies [8-11]. **(B)** Funnel plot of cementum regeneration with an obvious publication bias (Egger's test < 0.05) attributed to 1 point corresponding to 1 study [11].

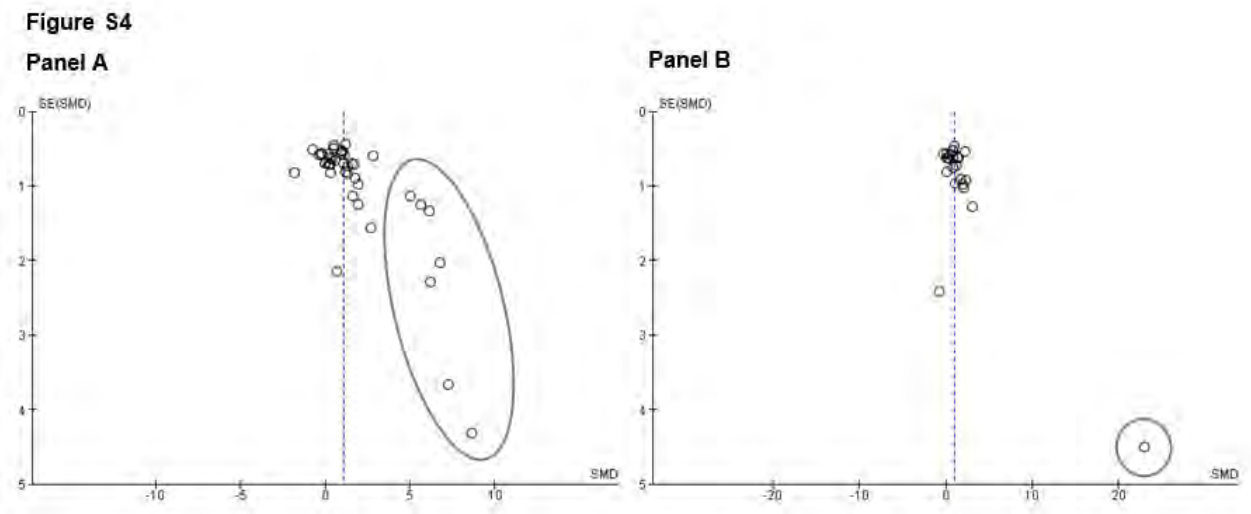


Table S1: Excluded studies are reported in this table with reason for exclusion. More details are supplied when the paper dealt with periodontal regeneration using stem cells, but did not include any data. The classification as “inappropriate” refers only to the status of the paper with regards to the objectives and inclusion criteria of this scoping study.

Study	Reason	Quote or explanation	
Akbay 2005	Inappropriate model	"The furcation was filled with a periodontal graft". No ex-vivo culturing step.	[12]
Block 2010	Inappropriate model	No periodontal defects but bone reconstruction after dental extraction	[13]
Borrego 2009a	Inappropriate model	No *ex-vivo* culturing step	[14]
Borrego 2009b	Inappropriate model	No *ex-vivo* culturing step	[15]
Duailibi 2008	No data to include		[16]
Flores 2008b	Inappropriate model	Subcutaneous model	[17]
Han 2010	Inappropriate localization	"Implanted into twelve 6 week old severe combined immunodeficient mice"	[18]
Hasegawa 2006	No data to include	Periodontal regeneration study after periodontal defect. Conclusion: transplanted BMSC, previously stimulated with *in-vitro* FGF-2, could survive and differentiate into periodontal tissue-composing cells, resulting in enhancement of periodontal tissue regeneration in class III furcation defects induced on female beagle dogs.	[19]
Inoue 1993	Inappropriate model	"Pouches were made in the rectus abdominis"	[20]
Ji 2013	Inappropriate model	“implanted into the peritoneal cavities of 8-wk-old nude mice”	[21]
Katayama 2006	Inappropriate model	No *ex-vivo* culturing step	[22]
Kawaguchi 2005	Japanese language		[23]
Kawaguchi 2008	Japanese language		[24]
Kim 2010	Inappropriate model	No cell seeding	[25]
Langenbach 2013	Review		[26]
Li 2008	Chinese language		[27]
Li 2010	Chinese language		[28]
Liu 2011	Inappropriate model	Critical size alveolar bone defect	[29]
Lu 2004	Chinese language		[30]
Ma 2008	Inappropriate model	Implanted into dorsal subcutaneous area of athymic mice	[31]
Marei 2005	Inappropriate model	No periodontal defects but bone reconstruction after dental extraction	[32]
Marei 2009	Inappropriate model	Peri-implant defects	[33]
McAllister 2011	Inappropriate model	No culturing step for cells	[34]
Modino 2005	Inappropriate model	"Surgically implanted into the soft tissues of the diastema region of the maxilla of adult mice"	[35]
Murano 2006	Inappropriate model	No *ex-vivo* culturing step	[36]
Neamat 2009	Inappropriate model	*In-vitro* study	[37]
No author 2007	Editorial paper		[38]
Ou 2000	Chinese language		[39]
Ou 2002	Chinese language		[40]
Reichert 2011	Inappropriate model	No *ex-vivo* culturing step	[41]
Ribeiro 2010	Inappropriate model	Peri-implant defects	[42]
Ribeiro 2012	Inappropriate model	Peri-implant defects	[43]
Seo 2004	No data to include		[44]
Song 2009	Inappropriate localization	Femoral condyles	[45]
Song 2012	Inappropriate localization	"Skin incisions were made on the dorsal surface of each mouse"	[46]
Takedachi 2013	No data to include		[47]
Tobita 2008	No data to include	Periodontal regeneration study after periodontal defect. Conclusion: eight weeks after Adipose Derived Stromal Cells (ADSC) implantation, regenerated bone and periodontal tissues were observed (cementum-like structures, alveolar crista and periodontal-like structure perpendicularly between cementum and alveolar bone). The author concluded human ADSC may be useful in periodontal tissue regeneration.	[48]
Uematsu 2013	Inappropriate localization	"Implanted into the subcutaneous tissue of nude mice"	[49]
Xu 2006	Chinese language		[50]
Yang 2010	Review		[51]

Yang 2012	Inappropriate localization	"Implanted into the dorsum of immunodeficient mice"	[52]
Yokoi 2007	Inappropriate model	Implanted subcutaneously	[53]
Yoshida 2012	Review		[54]
Young 2002	No data to include	Only mention "cementum like mineralized tissues"	[55]
Young 2005a	No data to include		[56]
Young 2005b	Inappropriate model	Implanted in the omenta	[57]
Zhang 2009	Method paper	Method used in Abukawa 2009	[58]
Zhang 2012	Inappropriate model	Osseous model only	[59]
Zuolin 2010	Hypothesis		[60]

Table S2: The data describes characteristics of included studies dealing with periodontal regeneration in animals. Conference proceedings were not included in this table. One study could be included in multiple groups (foreshadowing quantitative data analysis).

All studies	43 (100%)	All studies	43 (100%)	All studies	43 (100%)
Periodontal defects		***Cells sources***		***Outcomes***	
Fenestration defects	15 (35%)	From dental tissues	24 (56%)	Histological	39 (91%)
Infra-bony defects	14 (33%)	Periodontal ligament	23 (54%)	Radiological	6 (14%)
Furcation defects	15 (35%)	Dental pulp	2 (5%)	Clinical	16 (37%)
		Peri-apical follicle	1 (2%)	Biological	2 (5%)
		Primary follicle	1 (2%)	Safety	27 (63%)
		From gingival tissues	2 (5%)		
		From oral bone tissues	4 (9%)		
		From extra-oral bone marrow	10 (26%)		
		ES/iPS	4 (11%)		
		Other sources	5 (12%)		
		Genetically modified	11 (26%)		
Defect generation		***Grafts***		***Randomization***	
Mechanical (bur)	32 (74%)	Autologous	27 (63%)	Yes	21 (49%)
Bur with induction of inflammation	9 (21%)	Allogeneic	6 (14%)	No	22 (51%)
Orthodontic	3 (7%)	Xenogeneic	12 (28%)		
Animal model		***Delivery***		***Country***	
Dogs	21 (49%)	Bone substitute	9 (21%)	North America	7 (16%)
Rabbits	1 (2%)	Cell sheets	10 (23%)	South America	3 (7%)
Porcine	6 (14%)	Enamel Matrix Derivative	2 (5%)	Europe	6 (14%)
Rodents	14 (33%)	Plasma Rich Platelet, blood coagulum	4 (9%)	Asia	22 (51%)
Baboon	1 (2%)	Collagen, gelatin or hyaluronic acid	21 (49%)	Middle East	5 (12%)
		Polymers	10 (23%)	Australia	3 (7%)
		Guided tissue regeneration	13 (30%)		
		None	4 (9%)		
Gender					
Male	15 (35%)				
Female	12 (28%)				
Not reported	17 (40%)				

Table S3: Characteristics of each included animal study. Abbreviations: ADSCs – Adipose Derived Stromal Cells; BMP-2 - Bone Morphogenetic Protein 2; BMSCs – Bone Marrow Stromal Cells; CHX - Chlorhexidine digluconate; DBCB - Deproteinized Bovine Cancellous Bone; BOP – Bleeding On Probing; CAL – Clinical Attachment Level; DPSCs – Dental Pulp Stromal Cells; EMD – Enamel Matrix Derivative; ePTFE – Polytetrafluoroethylene; HA – Hydroxyapatite; IM – Intramuscular; iPS – Induced Pluripotent Stem cells; NA – Not applicable; NR – Not reported; PDLSCs – Periodontal Ligament Stromal Cells; PF127 – Pluronic F127, PGA – Polyglycolic acid; PPD – Periodontal Pocket Depth; TCP – Tricalcium phosphate

	Participants	**Periodontal defects**						**Groups**	**Outcomes**		**Conclusion**	**Score**
	Age *Gender* *Animal genus* *Country*	*Way of inducing* *Type of defects* *Description of defects*	*Number of defects*	*Type of cells*	*Type of graft* *Passage*	*Carrier* *Antibiotics or antiseptics after cell graft*	*Total duration* *Timepoints* *Study design* *Randomization*	*Experimental groups* *Control groups*	*Clinical, radiological or histological parameters*	*Biological parameters* *Societal and patient outcomes*	*Main conclusion according study author*	%
Akizuki [61] 2005	3 years Female Beagle dog Japan	Mechanical Fenestration defects Dehiscence defects were surgically created on the mesial roots of bilateral mandibular first molars (5x5 mm) using a burr. Cementum was curetted and cell sheet was applied.	Ten defects were generated on 5 dogs (2 by dog).	PDLSCs were isolated from mandibular premolars in each dog.	Autologous Explant method Passage 4 to 6	PDLSC single-layered cell sheet was prepared using a temperature-responsive cell culture dish. When cells reached confluence, culture medium was enriched with ascorbic acid. Hyaluronic acid sheet was added to reinforce. Penicillin G was administered IM to all dogs for 3 days. Plaque control with chlorhexidine digluconate (CHX) was used for 8 weeks.	8 weeks None Split-mouth No but histology examiner was blinded to the nature of treatment group	PDLSC cell sheet with hyaluronic acid carrier Hyaluronic acid carrier only	Defect height (mm) New cementum (mm) New bone (mm) Connective tissue attachment (mm) Root exposure (y/n) Acute inflammation (y/n) Periodontal healing with bone, cementum and periodontal ligament (y/n)	None Post-operative complications (y/n) Infection (y/n)	Using PDLSC sheets, periodontal tissue healing (bone, ligament and cementum) was achieved in 3 out of 5 defects, but only 1 in control group. Amount of cementum was also significantly increased.	75.9%
Chen [62] 2008	Adult Male New Zealand white rabbit Taiwan	Mechanical Fenestration defects Bilateral trans gingival periodontal defects were surgically created over the palate. Root surfaces were curetted.	Twenty-four defects were created and randomly assigned to four treatment groups. 2 defects for each of the 12 rabbits.	BMSCs were isolated from rabbit iliac crest. Experimental cells were transfected with E1-deleted adenovirus BMP-2. Control cells were transfected with adenovirus β-galactosidase containing the Lac-Z gene.	Autologous NA NR	BMSC were mixed with Pluronic F127 (PF127) and the construct was solidified into gel form in incubator. Parenteral injections of antibiotics performed for 3 days.	6 weeks None Split mouth Yes, defects randomly assigned.	Implantation of adenovirus-mediated BMP-2 gene-infected BMSC and PF127 Implantation of adenovirus-mediated βgal gene-infected BMSC and PF127 Implantation of BMSC and PF127 Implantation of PF127 only	Bone volume (mm^3) before, after, after-before Ankylosis (y/n)	None Adverse reactions (y/n) Infection during the healing (y/n)	Cells engineered to express the BMP-2 gene regenerated not only cementum with Sharpey's fiber insertion, but also statistically significant quantities of bone. Ex-vivo gene transfer may provide slower BMP-2 release increasing cementogenesis.	72.4%
Chung [63] 2011	Adult Male Beagle dog Taiwan	Mechanical Class III furcation defects Bilateral mandibular trans-gingival periodontal defects were created over the premolar area 4mm below cemento-enamel junction. Roots were curetted.	Nine dogs were used and two defects generated in each dog.	BMSCs were obtained from bone marrow aspiration. Cells were infected with adenoviral vector BMP-2 seven days before surgery.	Autologous NA NR	Carrier was a Pluronic F127 solution, BMSCs were mixed with it and allowed to solidify inside incubator. Parenteral antibiotics were injected for 3 days.	8 weeks None Split-mouth Yes, defects were randomly assigned	advBMP-2 infected BMSCs, with PF127 carrier Not infected BMSCs, with PF127 carrier	Total volume of regenerated bone (mm^3) Ankylosis (y/n)	None Adverse reaction (y/n) Uneventfully healing (y/n)	The use of ex-vivo BMP-2 engineered autologous MSCs allowed significant increase of bone volume and boosted periodontal regeneration apparatus compared to non-infected MSCs.	62.1%
Ding [8] 2010	6 to 8 months Female Wuzhishan inbred miniature	Mechanical Three-wall infra-bony defects Alveolar bone was removed using a surgical burr to create defects in	15 minipigs were used, 2 defects per minipig. These defects were randomly	PDLSCs were obtained from female Wuzhishan miniature minipigs and from male Guizhou	Autologous Allogeneic Enzymatic digestion Passage 3-4	On confluence, PDLSCs were cultured with hydroxyapatite/tricalcium to obtain cell sheets. Defects were	12 weeks None Split-mouth Yes	Autologous PDLSCs Allogeneic PDLSCs Autologous fibroblasts Control group: initial	Pocket depth (mm) Bone regeneration (%) Changes in periodontal depth (mm)	Percentage of CD3+, CD4+ and CD8+ cells (%) For allogeneic group: rejections (yes/no)	A sheet of minipig PDLSCs could repair allogeneic periodontal bone defects without immunological	62.1%

	minipig China	the mesial region of the maxilla and mandibular first molars (3 mm width, 7 mm length, and 5 mm depth).	assigned to five groups.	minipigs.		then covered with gelatin membranes. All animals received amoxicillin and metronidazole for 7 days, and mouth rinses of CHX for 5 days.		periodontal therapy only Transplantation of HA/TCP scaffolds and gelatin membrane covering only	Changes in clinical attachment loss (mm) Changes in recession (mm)		rejection.	
Dogan[64] 2002	Adult NR Beagle dog Turkey	Mechanical Class II furcation defects Lesions were induced removing 5mm of coronal bone and 2mm inter-radicular bone. Root surfaces were curetted.	Two defects for control side and 2 defects for experimental side were generated in one dog	PDLSCs were obtained from biopsies of *in-vivo* regenerated ligament tissue on Teflon membranes previously generated	Autologous Explant method Passage 4	Cells were adjusted in 0,5ml of blood clot before being inserted into periodontal lesions Irrigation of CHX for 4 weeks.	42 days None Split-mouth Not randomised	Blood clot with cells Blood clot without cells	New bone (mm/%) New attachment (mm/%) New cementum (mm/%) Extent of connective tissue (mm/%) Defect length (mm/%) Epithelial downgrowth (mm/%) Resorption (y/n)	None Post-operative complications (y/n)	Seeding of cells derived from regenerated periodontal ligament into furcation defects, promoted bone formation and connective tissue attachment on newly formed cementum.	60.0%
Dogan[65] 2003	Adult NR Dog Turkey	Mechanical Class II furcation defects Lesions were induced removing 5mm of coronal bone and 2mm inter-radicular bone. Root surfaces were curetted.	4 defects were created in one dog.	PDLSCs were obtained from incubating biopsies obtained from underneath the Teflon membranes, previously inserted into defects 4 weeks before experiment	Autologous Explant method Passage 4	Cells were mixed with autologous blood, centrifuged to obtain blood coagulum after clot formation. Irrigation of CHX for 4 weeks.	42 days None Split-mouth No	Defects with blood coagulum seeded cells Defects without cells (it was not reported if blood coagulum was transplanted)	Defect length (mm) Amount of bone fill (mm and %) Amount of cementum formation with connective tissue attachment (mm and %) Amount of connective tissue adaptation on the root surface without new cementum formation (mm and %) Epithelial down growth (y/n)	None Uneventfully periodontal healing (y/n)	Successful results were obtained in engineering periodontal tissues with compatible scaffolds and PDLSCs.	58.3%
Duan [10] 2011	8 weeks Male Nude mice China/USA	Mechanical Fenestration defects The buccal mandibular bone overlying the first molar was removed with spurs (2x1.5mm²). Roots were denuded of periodontal ligament, cementum and superficial dentine.	One defect was generated per mouse, 24 mice were used, and 4 mice were included in each group.	Induced pluripotent mouse stem cells (iPS) from human foreskin fibroblasts	Xenogeneic NA NR	Cells were seeded or not into apatite-coated silk fibroin scaffolds. Not reported.	24 days 12 and 24 days Parallel design No	Emdogain© and silk scaffold seeded with iPS. Scaffold group: silk scaffold without Emdogain© and without iPS. Control group: with Emdogain© and silk scaffold without iPS.	New bone area (%)	None None	Mouse iPS cells combined with EMD could greatly enhance the repair of mouse periodontal defects, by promoting the formation of cementum, alveolar bone, and a normal ligament.	75.9%
Fawzy El-Sayed [7] 2012	18±1 months 1 female and 7 male, castrated Minipigs Germany/Egypt	Inflammatory Two-wall infra-bony defects Cementum, periodontal ligament and alveolar bone were removed using surgical burrs to create defects in mesial regions of mandibular molars and premolars (3 mm x 7 mm x 5 mm). Silk sutures were left around cervical part of the teeth during 4 weeks to induce chronic inflammation.	Six defects were generated for each of the 8 minipigs.	Gingival marginal collars were surgically excised from the right canines of each animal to obtain gingival margin-derived progenitor cells	Autologous Explant method Passage 3 Cells were CD14+ (0.84%), CD34+ (1.24%), CD45+ (1.88%), CD31+ (98.41%), CD117 (97.83%), STRO1+ (37.61%).	Two types of carriers were used: one inorganic (porous matrix of deproteinized bovine cancellous bone or DBCB) and one organic (collagen scaffold). A collagen membrane was used to cover defects. Not reported, only Carprofen (Pfizer) was provided.	12 weeks None Split-mouth Randomization of sites using randomly permuted blocks. Random sequence was allocated and concealed until data acquisition and statistical evaluation. Examination was performed by a well-trained and blinded periodontist.	Group a: SRP + Cells + DBCB + Collagen membrane Group b: SRP + Cells + Collagen scaffold + Collagen membrane Group c: SRP + DBCB + Collagen membrane Group d: SRP + Collagen scaffold + Collagen membrane Group e: SRP Group f: No treatment	Clinical attachment level (mm) Periodontal pocket depth (mm) Gingival recession (mm) Bleeding on probing (%) Periodontal index RDV (Defect volume) HAL (Distance from the notch to the coronal extension of the periodontal attachment) JE (The length of the junctional epithelium - mm) CTA (Distance from the apical extension of the junctional epithelium to coronal extension of cementum - mm) Root resorption (y/n) Ankylosis (y/n)	None None	Gingival margin-derived stem/progenitor cells showed significant periodontal regeneration in adjunction to biocompatible scaffolds. This minimally invasive technique may be a valuable and practical alternative of cells with no need to sacrifice a tooth.	86.2%
Flores [66] 2008	5 weeks Male Athymic rats Japan	Mechanical Fenestration defects Cortical bone overlying the first and the second mandibular molars	Twenty rats for control groups, 20 rats for experimental group were used.	Human PDLSCs isolated from premolars.	Xenologous Explant method Passage 3 to 5	PDLSCs were cultured onto temperature-responsive culture dishes in presence or absence of osteogenic differentiation	5 weeks 3 to 5 weeks Parallel No	Experimental group received human PDLSC cell sheet cultured with osteogenic differentiation medium	Cementum regeneration rate (%) Newly formed cementum thickness (µm) Vertical ligament regeneration rate	None None	Transplantation of human PDLSC sheet treated with osteogenic medium regenerated better than	62.1%

		was removed. Periodontal ligament, cementum and superficial dentin were curetted. Roots were conditioned with EDTA and lubricant gel.				medium. PDLSCs were placed on the root defect and covered with a non-absorbable GORE-TEX membrane. Not reported.		Experimental group received human PDLSC cell sheet cultured in the absence of osteogenic differentiation medium None	(%) Regeneration of new cementum-like tissue (y/n) Formation of cementum-like structures and attachment of fibers (y/n) Signs of resorption (y/n)		PDLSC sheet without osteogenic medium, including attached Sharpey's fibers and periodontal ligament fibers.	
Grimm [67] 2011	10 weeks NR Athymic nude rats Germany	Mechanical Three-wall infra-bony defects Artificial bone defects were prepared on test and control sides at the level of the distal roots of first molars (2.5x2.5x2mm^3).	Twenty-two defects were generated: in each of 16 rats there was a positive control side and a test side. One rat served as a negative control. 5 rats were excluded because they died post-operatively	Human PDLSCs were isolated from patients with periodontal disease by minimally invasive surgery.	Xenologous NR NR	PDLSCs were first suspended into osteogenic differentiation medium before being seeding into collagen sponge scaffolds. Doxycycline was administered orally for 14 days.	2, 6 and 8 weeks 8 weeks Split-mouth No	Defects with collagen sponges and with cells (test side) Defects with collagen sponges and without cells (positive control) Defects without collagen sponges and without cells (negative control)	None	None Post-operative complications (y/n)	Human adult PDLSCs transplanted into an athymic rat model were able to regenerate periodontal tissue.	40.0%
Guo[68] 2013	3 months NR Rats Sprague Dawley China	Mechanical Three-wall infra-bony defects Alveolar bone was removed using surgical burrs to create experimental periodontal defects in the mesial region of the maxillary first molars (1 mm width, 4 mm length, and 2 mm deep). Roots were treated with EDTA before cell transplantation.	Two defects were generated per rat, but the total number of rats is unknown.	PDLSCs isolated from the middle third of the extracted molar root.	Allogeneic Explant method NR	PDLSCs were cultured with medium containing vitamin C (to increase extra-cellular matrix production). Formed cell sheets (mono or multi-layered) were transferred into 10ml conical polypropylene tube, cultured and harvested after 10 days. Not reported.	6 weeks None Split-mouth No but all procedures were performed blindly	Multi-layered cell pellet Mono-layered cell pellet None	Regeneration of mineralized tissue (y/n)	None None	By increasing the extra-cellular deposit matrix, the strength of the multi-layer cell pellet scaffold was reinforced. This 3-D cultivation system may enhance the mineral capability of PDLSCs, allowing complete reconstruction of physiological architecture of cementum/periodontal ligament complex.	55.2%
Hasegawa [69] 2005	12 weeks Female Athymic rats (Fischer 344) Japan	Mechanical Two-wall infra-bony defects Bone was removed to a level 2mm apical from the marginal bone crest and 2mm at the mesial side of the mesial root using a chisel. Root was curetted to remove periodontal ligament and cementum. Then cell sheets were applied.	Two defects per rat on 6 rats were created; right side was the experimental side and left side was the control side. Half of the defects were analyzed at 1 week, half at 4 weeks.	Human PDLSCs from a third molar toot of a 20-year-old patient with no periodontitis.	Xenogeneic Explant method Passage 3 to 5	PDLSC cell sheets were prepared using temperature-responsive cell culture dishes. When cells reached confluence, culture medium was enriched with ascorbic acid. Not reported.	4 weeks 1 and 4 weeks Split-mouth No	Cell sheets transplantation Without cell transplantation	Gingival tissue detachment (y/n)	None Post-operative complications (y/n)	PDLSC sheets were able to stimulate regeneration of periodontal ligament tissues in a xenogeneic model using human stem cells in athymic rats.	62.5%
Han [70] 2013	Adult Female Sprague Dawley rats Australia	Mechanical Fenestration defects Defects were created unilaterally on the right-hand side of the mandible, around first and second molars. Roots were denuded.	One defect was created per animal, 36 rats were used; 3 per group and time point.	PDLSCs derived from one rat were used.	Allogeneic Enzymatic digestion NR	PDLSC were labelled with BrdU and 10^6 PDLSCs were mixed with absorbable gelatin sponge, spotted with α-MEM + 10% allogeneic serum. Implants were clotted with fibrinogen/thrombin. Not reported.	28 days 7, 14, 21 and 28 days Parallel Yes without details. A single calibrated examiner was blinded to the study groups.	Cells with collagen sponge Collagen sponge without cells Untreated	Percentage of the total area of newly formed mineralized tissue filling the defect (%) Mean length of new bridging bone (%) Length of new cementum (mm)	None Post-operative complications (y/n) Tumor formation (y/n)	Allogeneic PDLSCs were able to repair periodontal fenestration defects. Treatment period should be between 14 and 21 days (for optimal quantitative analysis in rodents.	82.8%
Hovey[71] 2006	Adult Female Baboon USA	Inflammatory Class III furcation defects Through-and-through furcation and interproximal defects from the fornix of furcation to reduced alveolar crest were realized around the first and second mandibular	Four defects per baboon were realized in 6 baboons.	DGs: three-dimensional, allogeneic, human neonatal undifferentiated dermal fibroblasts.	Xenogeneic NA NA	EDTA was applied first onto roots of experimental groups. EMD was applied mixed with polyglycolic acid. DGs were stocked at -70°C before use. Not reported. Only analgesia was provided.	5 months Split-mouth Yes, with randomization table. The single examiner was blinded.	DG alone DG and EMD OFD: open flap debridement OFD with Emdogain	<u>Mid-furcation</u> New bone height (mm) New connective tissue (mm) New epithelium (mm) <u>Intraradicular root surfaces</u> New cementum (mm) New attachment apparatus (mm)	None None	DG groups demonstrated less favorable responses: this could be explained by immune host response (xenotransplantation)	89.7%

		molars. Root surfaces were curetted; a cotton pellet attached to an orthodontic wire was placed for 8 weeks.							New connective tissue attachment (mm) New epithelial attachment (mm)			
Hynes [72] **2013**	NR NR Athymic nude rats Australia	Mechanical Fenestration defects Defects were created unilaterally on the right-hand side of the mandible, around first and second molars. Roots were denuded.	One defect was created per animal. Number of animals was unclear. We believed 6 rats for cell group and 3 rats for each control.	Human foreskin-derived iPS were obtained from The National Stem Cell Foundation of Australia.	Xenogeneic NA NR	Cells were differentiated by MSC media for 2 weeks. Cells were clotted with a fibrinogen/thrombin mix. Not reported.	2 weeks None Parallel No but slides were chosen by a single blinded assessor.	Cells with clotting factors Clotting factors alone Defect only	Total area of new bone formed (%) Bone bridging (y/n)	None None	iPS-MSC cells have the capacity to increase the amount of regeneration and newly formed mineralized tissue in periodontium.	51,7%
Iwata [73] **2009**	NR Male Beagle dog Japan	Mechanical Three-wall infra-bony defects Defects were created (5x5x4) surgically on the mesial side of bilateral mandibular first molars. Root cementum was removed and conditioned with EDTA.	Four dogs were used, 2 defects were generated per dog.	PDLSCs were obtained from mandibular premolars	Autologous Enzymatic digestion Passage 3	PDLSCs were seeded onto temperature-responsive culture dishes and cultured medium was supplemented with ascorbic acid. When cells reached confluence, culture medium was changed to osteogenic medium. Tri-layered sheets were formed using wet sheets of woven polyglycolic acid (PGA). Defects were filled with porous βTCP. Penicillin G for 3 days and topical application of CHX twice a week for 6 weeks.	6 weeks None Split-mouth No but parameters were measured by two calibrated examiners who were blinded to the experimental conditions	Defects were filled with tri-layered PDLSC sheet supported by PGA sheets, and βTCP. Defects were filled with PGA sheets, and βTCP.	Junctional epithelium (mm) Bone regeneration (%) Cementum regeneration (%) Periodontal score (1 to 5) Ankylosis (y/n) Root exposure (y/n)	None Post-operative complications and adverse reactions (y/n)	Tri-layered PDLSC sheets were successfully used to induce true periodontal regeneration, including more alveolar bone and more cementum than control group, with well-oriented fibers.	72.4%
Jiang [74] **2010**	NR NR Beagle dog China	Inflammatory Class III furcation defects Defects were created surgically at the mandibular premolars using diamond burrs. Alveolar bone was removed creating a horizontal pattern (3x4mm). Defects were filled with gutta-percha to promote inflammation process. Root surfaces were curetted and recovered with ePTFE during 6 weeks.	Six defects were generated per dog, 4 dogs in total. The 24 defects were allocated to three groups.	Small pieces of periosteum were harvested from the mandible of each dog.	Autologous Explant method Passage 3	Granulous porous ceramic β-TCP was used to fill defects. No oral hygiene was instituted.	12 weeks None Split-mouth Yes, defects were randomly divided into 3 groups.	Group A: e-PTFE with β-TCP and periosteal cells Group B: e-PTFE with β-TCP only Group C: ePTFE alone	Total defect length (mm) New cementum formation (mm/%) New periodontal formation (mm/%) Total defect area (mm²) New bone area (mm²/%) Soft tissue area (mm²/%) Non-filled area (mm²/%)	None Adverse events (y/n)	Autogenous periosteal cells with βTCP were able to promote periodontal tissue regeneration in class III furcation defects better than β-TCP alone.	72.4%
Kawaguchi [75] **2004**	12 to 20 months Female Beagle dog Japan	Mechanical Class III furcation defects Defects were created surgically at second, third and fourth premolars. Height from the cemento-enamel junction to the alveolar crest was reduced to 4mm. Roots were curetted to remove periodontal ligament and cementum	All four groups of the MSC-collagen gel and the atelocollagen alone were transplanted in all 12 dogs.	BMSCs from the iliac crest of beagle dogs were maintained in a medium with FGF-2.	Autologous NA Passage 3	BMSCs were stored into liquid nitrogen until use, and mixed with atelocollagen (2% type I collagen extracted from bovine calf skin by pepsin digestion). Teeth were brushed, and swabbed with povidone iodine.	2 weeks 1 and 2 weeks NR NR	Atelocollagen and $2x10^6$ BMSC Atelocollagen and $5x10^6$ BMSC Atelocollagen and $1x10^7$ BMSC Atelocollagen and $2x10^7$ BMSC Atelocollagen only	New cementum lenght (%) New bone area (%) Bone ankylosis (y/n) Root resorption (y/n)	None None	More periodontal regeneration, cementum, periodontal ligament, alveolar bone, was observed in BMSC-atelocollagen group compared to control group.	72.4%
Khorsand [76] **2013**	1 à 2 years Male Mongrel dogs Iran	Inflammatory Three-wall infra-bony defects Alveolar bone was removed using surgical burr to create defects bilaterally in the mesial of the mandibular first premolar: 3mm	In total 20 defects were created in 10 dogs.	DPSCs came from 2 maxillary premolar teeth from each dog.	Autologous Enzymatic digestion Passage 3	DPSCs were mixed with Bio-Oss®, bovine bone. All animals received cefazolin and tramadol IM during 3 days, daily mouthwash of CHX for 2 weeks.	8 weeks None Split-mouth Yes, defects were randomly attributed.	Cells with bovine bone Bovine bone only	Cementum formation (mm) Cementum formation (y/n) Alveolar bone formation (mm) Alveolar bone formation (y/n) Periodontal ligament formation (mm)	None Post-operative complications, suppuration, infection (y/n)	Use of DPSCs is promising for periodontal regeneration. This study showed significant reconstruction of cementum and ligament in addition to regeneration of alveolar	75.9%

		width, 8mm length, and 5mm depth. Roots were curetted. Silk ligament was sutured around the cervical portion of the first molars for 1 month. A scaling and root planning was performed before transplantation.							Periodontal ligament formation (y/n) Gingival recession (y/n) Root exposure (y/n) Residual particles (y/n)		bone.	
Lang [77] **1998**	Adult (4 to 8 years) Female Minipigs Germany	Inflammatory/Orthodontic Two-wall infra-bony defects and grade II furcation defects Defects were created with orthodontic elastics and removed after 62 days. Metal wires were also bonded to the buccal aspect of the defect with their apical ends located at the entrance of the furcation. Root surfaces were curetted.	Eleven minipigs were used and 168 defects were generated in total. Each treatment group of 10 and 30 days consisted of 3 animals with 3 interdental defects and 3 furcation defects. For the 90 day group, 5 animals with 8 interdental defects and 8 furcation defects.	PDLSCs were obtained from middle third of the root of the removed premolars. Alveolar bone cells were also obtained.	Autologous Enzymatic digestion Passage 1	Cells were pelleted into the bone gelatin with serum-free cell culture medium cooled to 4°C to have a gel-like material. Not reported.	90 days 10, 30 and 90 days Split-mouth NR	ABS: flap surgery with bone gelatin, alveolar bone cells and ePTFE PDLSC: flap surgery with bone gelatin, PDLSC and ePTFE BG: flap surgery with bone gelatin and ePTFE NBG: flap surgery with ePTFE to cover FS: Flap surgery only NT: No treatment	Probing depth reduction (mm) Gingival recession (mm) Periodontal regeneration (%) Connective tissue attachment (%) Connective tissue adhesion (%) Epithelial attachment (%)	None None	Study suggests that replantation of cultured cells leads to formation of new cementum and bone, then to a new attachment. Cells stabilize tissue formation and prevent epithelial downgrowth.	69.0%
Lekic[78] **2001**	NR Male Sprague Dawley rats Canada	Mechanical Fenestration defects Periodontal window wounds were created by removing alveolar bone and periodontal ligament over the mesial root of the first molar.	Twelve rats were used to create one defect per rat	Precursor cells from root, bone and endosteal spaces of alveolar bone were obtained from 6-8 week old Sprague Dawley male rats and from transgenic male mice expressing β galactosidase	Allogeneic and Xenogeneic Explant method Passage 2	Cells were mixed with a collagen gel, prepared by mixing chilled bovine dermal type I collagen and 0,1M NaOH. Before incubation in gel, cells were loaded green fluorescent latex beads. Not reported.	2 weeks 1 and 2 weeks Parallel No	β galactosidase expressing precursor cells, seeded in collagen gel Fluorescent beads loaded precursor cells , seeded in collagen gel Control group was untreated	Normal periodontal width (%) Newly formed alveolar bone (%) Labelled cells in regenerating alveolar bone (%) Labelled cells in regenerating periodontal ligament (%)	None None	Transplantation of cells loaded with fluorescent beads is a useful method for assessing the fate and differentiation of periodontal cells in vivo. Both precursor cells promoted regeneration of alveolar bone.	58.6%
Lekic[79] **2005**	NR Male Sprague Dawley rats Canada	Orthodontic Two-wall infra-bony defects One day before cell transplantation, defects were created inserting separating elastic between the first and the second mandibular molar. Cells were provided using a dental probe.	Sixty rats were used; one defect was created per rat.	GFP+ ES cells were used Precursor from the root-related and bone-related compartments, as well the endosteal spaces of the alveolar bone, were obtained from Lac-Z-positive TgR mice BMSCs were collected from femur	Xenogeneic NR Passage 4 to 5	No carrier Not reported.	3 days 1 and 3 days Parallel No	Orthodontic tooth movement (OTM) + PDLSC OTM + BMSC OTM + ES OTM + xenogeneic skin fibroblasts OTM + No cells Cell transplantation alone Nothing	Periodontal ligament width (mm) Alveolar bone width (mm) Apoptotic cells (%)	None Immunological response to xenograft (y/n)	Transplanted periodontal ligament and bone marrow cells migrate systemically and follow a cyclical process of growth and development, and differentiate to contribute to periodontal regeneration.	44.8%
Li [11] **2009**	Adult Female Beagle dog China	Mechanical Fenestration defects The fenestration defects (5x5mm) were established 5mm apical to the cemento-enamel junction using dental bur. Root surfaces were denuded of PDL, cementum and superficial dentin.	26 teeth in 5 dogs were selected: 18 teeth for experimental groups, 8 teeth for control group.	BMSCs were isolated from femur bone marrow.	Autologous NA Passage 4	Cryopreserved or not cryopreserved BMSCs were seeded onto collagen membranes. Membranes were placed in contact with dental roots. Defects were covered with e-polytetrafluoroethylen (e-PTFE). Penicillin was administered IM for 4 days.	8 weeks None Split-mouth Yes with partial block design, and single examiner was blinded.	Cryopreserved BMSC Non cryopreserved BMSC Control group: collage membrane without cells	New bone (%) New cementum (%) New periodontal ligament (%)	None Post-operative infection (y/n)	Both freshly isolated and cryopreserved BMSC transplants induced significantly better periodontal regeneration with newly formed cementum, alveolar bone and periodontal ligament compared with the application of collagen scaffold alone.	82.8%

Liu [80] **2008**	12 months NR Inbred miniature pigs China	Inflammatory Two-wall infra-bony defects Alveolar bone was removed using surgical burr to create defects in the mesial region of the maxillary and mandibular first molars (3mm width, 7mm length, and 5mm depth). Silk ligament was sutured around the cervical portion of the first molars for 1 month.	48 defects were created in 12 animals (3 animals per group).	PDLSCs were isolated from minipig cuspids.	Autologous Enzymatic digestion Passage 3	PDLSCs were infected with recombinant retroviral vector with green fluorescent protein. PDLSCs were combined with HA/TCP and defects were covered with gelatin membranes. All animals received amoxicillin and metronidazole for 7 days, and mouth rinses of CHX for 5 days.	12 weeks 4 and 12 weeks Split-mouth Yes, defects were randomly attributed to three different treatment groups	PDLSC group with HA/TCP HA/TCP only No treatment group	Baseline and final pocket depth (mm) Baseline and final gingival recession (mm) Baseline and final attachment loss (mm) Lenght of regenerated alveolar bone (mm)	None None	It could be possible to use autologous PDLSC grafting to treat periodontitis in a miniature pig preclinical model.	79.3%
Mizuno [81] **2006**	Adult Female Hybrid dog Japan	Mechanical Class III furcation defects Alveolar bone defects were created at the root furcation of the fourth left mandibular premolars. Periodontal tissues were mechanically removed using dental drill. Defects were 3mm wide and 4mm high. Root was planed.	Six defects were created, one defect in each of the six dogs	Periosteum samples were peeled from the mandibular body of each dog.	Autologous Explant method NR	Cells were incubated until cells formed a sheet, then membranes were placed to pack around the exposed roots. Not reported.	3 weeks None Parallel Not randomized	With cell sheet Without cell sheet	Regeneration at the lateral surface of the roots (y/n)	None None	Periodontal defects at the root furcation and lateral root surface were successfully regenerated by periosteum cell grafting. Cell sheet acts as membrane structure and provides osteogenic potential.	45.8%
Nakahara [82] **2004**	Adult Female Beagle dog Japan	Mechanical Fenestration defects Defects were created 6mm apical to the marginal alveolar crest in the maxillary bilateral canines. Bone was removed with burrs and chisels. Roots were scaled to remove cementum.	Two defects were generated in each of 6 beagle dogs	PDLSCs were obtained bilaterally from lateral incisor teeth from each dog, then frozen until use.	Autologous Explant method NR	Collagen sponge scaffold was prepared from 80% type I and 20% type III atelocollagen. Cells were seeded and defects covered with expanded polytetrafluoroethylen (e-PTFE). Not reported.	4 weeks None Split-mouth Yes, defects were randomly implanted, and scoring was blinded	Cell suspension without fetal bovine serum seeded onto collagen sponge scaffold Collagen sponge scaffold without cells	Bone regeneration (mm) Cementum regeneration (mm) Inflammatory cells (y/n)	None Post-operative complications (y/n)	On the cell-seeded side, regeneration of the cementum was observed uniformly on the root surface. Autologous PDLSCs have the potential for in situ engineering of the periodontal tissues.	69.0%
Nayak[83] **2008**	6-8 weeks Male Sprague Dawley rats Canada	Orthodontic Two-wall infra-bony defects Tooth movement was initiated by placing elastic orthodontic ligature between the first and second mandibular molar.	Five animals were used per group (30 animals).	Precursor cells located in the endosteal spaces of the alveolar bone and from the periodontal ligament of transgenic male C57BL/6J B26 mice. Mice ES cells were GFP labelled	Xenogenous Enzymatic digestion for precursor cells Passage 4 to 5	None Not reported.	72 hours 24 and 72 hours Parallel design No	Tooth movement and lac-Z-positive precursor Tooth movement and ES cells No tooth movement and no cell transplantation Tooth movement and no cell transplantation	Ratio of transplanted cells compared to total number of cells (%) Transplanted cells expressing OPN, BSP, αSMA (%) Periodontal ligament width (mm) Alveolar bone width (mm)	None None	This study underlined the high degree of regeneration of the periodontium in transplanted PL cells compared with ES cells, likely because of the plasticity, level of differentiation, and tissue microenvironment of the PL cells.	62.1%
Nuñez [84] **2012**	One year old Male Beagle dog Spain	Inflammatory Three-wall infra-bony defects Defects were created measuring 3 mm wide and 4 mm deep. Orthodontic wire ligatures were placed to allow plaque accumulation and inflammation for one month.	Six defects were generated per animal, 4 dogs in total.	PDLSCs and Cementum-Derived Cells (CDCs) were isolated from mandibular premolars.	Autologous Enzymatic digestion Passage 4 to 5	Collagen sponge scaffolds were employed. Animals received amoxicillin (IM) for 7 days, anti-inflammatory for 3 days and topically applied CHX for 3 months.	12 weeks None Split-mouth Yes, treatments were randomized by the tossing of a coin. A well trained examiner blinded to the groups carried out the examination.	Sponges with PDLSC Sponges with CDC Control sponges with culture medium and without cells	Length of the coronal level of histological attachment (mm) Length of the connective tissue adhesion (mm) Length of the newly formed cementum (mm) Length of the newly formed bone (mm) Gingival margin to apical extent of the junctional epithelium (mm) Gingival margin to cemento-enamel junction (mm) Root resorption (y/n) Ankylosis (y/n)	None Post-operative complications (y/n)	CDC and PDLSC groups have significantly higher amounts of newly formed cementum and attachment than control group.	86.2%

Park [9] **2011**	10 months NR Beagle dog Korea	Inflammatory Fenestration defects Alveolar bone was removed with surgical burr to create apical involvement defects (3mm wide circumferential defects around the mesial roots of premolars). Roots were scaled. Defects were filled with a rubber base impression material to induce inflammatory response for 4 weeks.	Eight dogs were involved in this study, with a total of 32 defects.	PDLSCs Dental pulp stem cells Peri-apical follicular stem cell (PAFSCs) from immature molar tooth.	Autologous Enzymatic digestion Passage 2 to 3	None Animals received amoxicillin orally for 7 days, mouth washes with CHX for 5 days.	8 weeks None Split-mouth Yes, defects were randomly allocated.	PDLSC graft group Dental pulp stem cell graft group Peri-apical follicular stem cell group Positive control group: periodontal defect without cell seeding Negative control group: no defect but sham surgery	Periodontal pocket depth (mm) Gingival recession (mm) Attachment level (mm) Height of the new bone formation (mm) Volume ratio of the newly formed bone (%)	None Post-operative infection (y/n) Neoplasm formation (y/n) Toxic signs (y/n)	PDLSCs and PAFSCs generated new cementum and Sharpey's fibers as well as the alveolar bone. Authors suggested PDLSCs were the best candidates for regeneration, obtaining the best results with respect to the quality and the quantity of the regenerated tissues.	79.3%
Park [85] **2012**	NR NR Athymic rat USA	Mechanical Fenestration defects A single fenestration defect 3x2mm² was created on the buccal side of the mandible. The exposed roots of first molar teeth were curetted to expose dentin layer.	Forty-eight rats were enrolled in this study, one defect created per animal.	Human PDLSCs from 3 donors. Some of these cells were transfected with a recombinant adenovirus-encoding murine bone morphogenetic protein-7 (Ad-BMP7).	Xenologous NR Passage 4 to 5	Randomly-porous scaffolds and fiber-guiding scaffold structures were created using poly-ε-caprolactone solution in acetone. A thrombin/fibrinogen mix was added to cell-seeded scaffolds. Only analgesics were administrated for 1 day.	6 weeks 3 and 6 weeks Parallel No	PDLSC into randomly-porous scaffolds PDLSC into fiber guiding scaffolds Ad-BMP7-PDLSC into randomly-porous scaffolds Ad-BMP7-PDLSC into fiber guiding scaffolds None	Bone volume fraction Tissue mineral density (mg/cc) Ligament orientation (°)	None None	Cell transplantation using fiber guiding scaffold results in better organization of ligament and higher amount of bone regeneration than randomly-porous scaffolds.	51.7%
Simsek [86] **2012**	Young adult NR Mongrel dog Turkey	Inflammatory Class II furcation defects Lesions (5 mm x 2 mm directions) were created on the buccal surface of maxillary and mandibular premolars of both sides. Defects were filled with rubber impression paste for 3 weeks to prevent spontaneous repair and induce an inflammatory response.	Six lesions were generated by dog. In each dog 2 defects were used for each group.	BMSCs were obtained from iliac crest bone marrow aspirate.	Autologous NA NR	Plasma rich platelets (PRPs) were coagulated with calcium chloride, and mixed with BMSCs. Cortical bone particulates were also added into defects (ACB). Plaque control was achieved mechanically and by CHX for 2 weeks.	8 weeks None Split-mouth No, but examiners were blinded	BMSC with PRP Surgical procedure only Procedure with PRP alone Procedure with ACB alone Procedure with ACB and PRP	New cementum (%) New alveolar bone (%) Significant gingival recession (y/n) Root resorption (y/n) Ankylosis (y/n)	None Post-operative complications (y/n)	Periodontal regeneration with complete filling of class II furcation defects is obtained with ACB, ACB/PRPs and MSCs/PRPs; however, no group showed higher efficacy than another.	86.2%
Suaid [87] **2011**	1.46±0.18 years NR Beagle dog Brazil	Mechanical Class II furcation defects Created at the buccal aspect of maxillary third premolars, osteotomy was performed with diamond burrs and standardized (5mm x 2mm).	Two defects were created per dog (one for control, one for treatment), 7 dogs in total.	PDLSCs were obtained from bilateral mandibular second premolar and first molar from each dog. Cells were then frozen.	Autologous Enzymatic digestion Passage 2 to 3	Collagen sponge soaked with DMEM with or without cells, associated with an absorbable membrane (Gore-Tex) adapted to the defect. Plaque control was achieved mechanically and by CHX for 3 months. IM penicillin was given for 4 days.	3 months None Split-mouth Yes, defects were randomly assigned. Study was blinded.	Cell-seeded collagen sponge scaffold Collagen sponge scaffold without cells	Total defect length (mm) Total new cementum (mm) Periodontal regeneration (mm) Epithelium/connective tissue extension (mm) Ankylosis (mm) Defect area (mm²) Non-filled area (mm²) Soft tissu area (mm²) New bone area (mm²) Ankylosis (y/n) Healing (y/n)	None Post-operative complications (y/n)	PDLSCs in conjunction with guided tissue regeneration may promote periodontal regeneration in class II furcation defects in dogs.	82.8%
Suaid [88] **2012**	1,46±0,18 years NR Beagle dog Brazil	Mechanical Class III furcation defects Bilateral furcation defects were created on mandibular premolars, removing alveolar bone with chisels and burrs. Size was standardized at 5 mm in apico-occlusal direction. Roots were scaled and curetted to remove ligament and cementum.	Four defects were generated per dog, 7 dogs in total.	PDLSCs were obtained from mandibular premolars and molars.	Autologous Enzymatic digestion Passage 2 to 3	Collagen scaffolds (sponges) were applied into defects, and covered with bio-absorbable membranes (Gore-Tex). Plaque control was achieved mechanically and by CHX for 3 months. IM penicillin was given for 4 days.	12 weeks None Split-mouth Yes, defects were randomly assigned to groups	Collagen sponge scaffold with bio-absorbable membrane and cells Bio-absorbable membrane alone Collagen sponge scaffold with bio-absorbable membrane without cells Control group, surgery alone	Total defect length (mm) Tissue free defect length (mm) New cementum (mm) Periodontal regeneration (mm) Epithelium/connective tissue extension (mm) Total defect area (mm²) Non filled area (mm²) Soft tissue area (mm²) New bone area (mm²) Exposure of the furcation area	None Post-operative complications (y/n)	PDLSCs with bio-absorbable membrane promote more periodontal tissue regeneration (new cementum and new bone formation) than control groups.	75.9%

									(y/n) Exposure of the coronal part (y/n) Complete filling of defects (y/n) Ankylosis (y/n) Root resorption (y/n)			
Tan [89] **2009**	6-10 months Male Beagle dog China	Mechanical Class III furcation defects Defects 5 mm in height were created by osteotomy on the mandibular premolars. Roots were curetted to remove periodontal ligament and cementum.	Six defects were created for each of the 4 dogs employed in this study.	BMSCs were isolated from tibia bone marrow Half of the BMSCs were transfected to express bFGF gene	Autologous NA Passage 3	BMSCs and bFGF-modified BMSCs were suspended into sodium alginate solution, mixed with calcium chloride solution to form a calcium alginate gel, and transplanted into defects. Scaling was performed every week, plaque control was also achieved with CHX. Animals were given antibiotics for 3 days (streptocillin) and analgesics for 1 day.	6 weeks None Split-mouth Yes, defects were randomized.	BMSC transfected with bFGF gene Untransfected BMSC	Regeneration area (%) Height of newly formed bone (mm) TV (mm3) BV (mm3) BV/TV (%) Tb.Th (mm)	None Post-operative complications (y/n)	BMSCs transfected with bFGF may accelerate periodontal regeneration compared to BMSCs alone.	65.5%
Tobita [90] **2013**	9-10 months NR Beagle dog Japan	Mechanical Class III furcation defects Defects were created surgically at the bilateral second, third and fourth mandibular premolars. Height: 5mm. Roots were curetted to remove periodontal ligament and cementum	Eight animals were included, 48 defects in total. Each dog received all groups (bilaterally).	Inguinal fat pads were harvested from the eight dogs to obtain ADSC	Autologous Enzymatic digestion Passage 2	Autologous PRP was used to be mixed with ADSCs, and transplanted into defects. Not reported.	2 months 1, 2 months Split mouth No	ADSCs/PRP mix PRP only Non-implantation	Percentage of new bone area (%) Percentage of new cementum length (%)	None None	Combination of ADSCs and PRP had potential to obtain regeneration of periodontal tissues with normal architecture in canine model. This regeneration was achieved at 2 months and not 1 month.	72,4%
Tsumanuma [91] **2011**	NR Male Beagle dog Japan	Mechanical One-wall infra-bony defects Defects were created on mandibular premolars and molars bilaterally. Root cementum was curetted, and then treated with EDTA. Cell sheets were applied or not.	Six defects were created in each of 4 dogs, 3 attributed to the 3 transplantation groups and 1 for control group. Remaining defects were used for another study.	PDLSCs from mandibular premolars BMSCs from iliac crest APCs from mandibular periosteum	Autologous Enzymatic digestion Passage 3	Cells were seeded on temperature-responsive culture dishes, cultured in osteogenic medium for 5 days. Cell sheets were harvested and reinforced with sheets of woven polyglycolic acid (PGA). Procedure was repeated to build three-layered cell sheets. Before suturing, defects were filled with porous β- TCP mixed with 3% type I collagen. Animals received azithromycin for 3 days, mouth washes with CHX for 8 weeks.	8 weeks None Split-mouth No but examiners were calibrated and blinded to experimental conditions.	Cell sheets with BMSCs Cell sheets with PDLSCs Cell sheets with APCs Only PGA sheet was applied (without cell)	Newly formed cementum thickness (μm) Periodontal score (1 to 5) Bone regeneration ratio (%) Length of junctional epithelium (mm) Root resorption (y/n) Ankylosis (y/n) Root exposure (y/n)	None Post-operative complications (y/n) Infection (y/n)	Periodontal regeneration was highest in PDLSC group. There was more newly-formed cementum, more well-oriented PDL fibers and more alveolar bone than in the other groups.	75.9%
Van Dijk[92] **1991**	9 years NR Beagle dog Netherland	Mechanical Fenestration defects Defects were made at the buccal side of the lower second, third and fourth premolars on both sides of the jaw. Supporting bone was removed and periodontal ligament was scraped.	Six defects were created on 1 dog.	Periodontal ligament was removed from an upper premolar to obtain PDLSCs	Autologous Explant method Passage 5	None Oral hygiene was not performed.	4 months None Split-mouth No	PDLSCs were applied onto roots No cells were applied onto roots	Root resorption (y/n) New connective attachment (y/n)	None None	After 4 months the seeded root surfaces were almost completely covered with cementoblasts, whereas in controls, epithelial downgrowth could be observed.	33.3%

Yang [93] **2010**	8 weeks Male Sprague-Dawley rats Canada	Mechanical Fenestration defects The alveolar bone over the mandibular first molar roots was removed with a burr. The periodontal wound window was approximately 1x3mm. The exposed roots were denuded to remove periodontal ligament and cementum.	One defect was created per rat and 14 rats were used.	BMSCs from eight weeks old male GFP+ transgenic SD rats	Allogeneic No enzymatic digestion NR	BMSCs were cultivated on gelatin beads, and then implanted into defects. Pain control was achieved by buprenorphine and cyclosporin A was given subcutaneously.	3 weeks None Parallel design No	Filled with BMSCs expanded on gelatin beads Filled with gelatin beads only Defects without gelatin beads	New bone formation area (%) New ligament density of fibers 80-90° (#/100um) New ligament density of fibers 60° (#/100um) Cementum regeneration (%) TRAP positive cells (#/bead)	None Post-operative sequels (y/n)	Animals that received BMSCs regenerated significantly greater new bone and appropriately oriented periodontal ligament fibers, indicative of functional restoration.	65.5%
Yang [94] **2013**	5 months Female Miniature pigs Taiwan	Mechanical Class II furcation defects Osteotomy was done in the furcation area using burrs (4 mm wide, 5 mm deep and 3 mm horizontally). Polyester impression material was inserted into the defect to induce chronic inflammation for 4 weeks.	Twelve defects were generated in 6 pigs (2 defects per pig).	GFP-expressing porcine ES (pES) were used	Allogeneic NA Passage 43 to 45	10^6 pES/GFP+ cells were transferred onto a collagen matrix. Not reported.	12 weeks None Split-mouth Yes, defects were randomly assigned	Collagen matrix with cells Collagen matrix alone	PPD (mm) CAL (mm) Inflammation (y/n)	Fluorescence intensity Post-operative adverse effects Neoplasms	Results suggested that pES/GFP+ derived cells might migrate to remote tissue, especially tissue with a high turnover rate, via blood vessels. Using ES cells could improve the regeneration of periodontal furcation defects.	82.8%
Yu [95] **2013**	7 weeks NR Athymic nude rats Netherlands	Mechanical Three-wall infra-bony defects Created mesially at both maxillary first molars with a piezoelectric device. Roots were curetted to remove residual bone, periodontal ligament and cementum. Volume was about 2x2x2mm^3.	Twelve rats were used, total of 24 defects allocated according a "latin square".	Cells from periodontal ligament and gingiva of GFP-transgenic Sprague-Dawley rat were used.	Xenogeneic NR Passage 4	Cells were seeded into collagen sponge. Not reported.	6 weeks None Split-mouth Yes	Collagen sponge with PDLSC Collagen sponge with gingival cells Sponge only	Gingival inflammation on histology (y/n) Scaffold remnant (y/n) Root resorption (y/n) Bone and cementum regeneration (y/n) Relative new bone area Relative epithelial down-growth Relative functional ligament length	None Post-operative complications (y/n)	After 6 weeks, periodontal cells exhibited significant more functional bone and ligament. Gingival cells did not provide favorable effect.	75.9%
Wei [96] **2010**	6 to 10 months Male Beagle dogs China	Mechanical Class III furcation defects Mandibular premolars on both sides were selected. The furcation alveolar bone was removed from the buccal to lingual side, and a horizontal pattern was created using diamond burr. Roots were curetted to remove periodontal ligament and cementum.	Four dogs were used, 6 defects were created per dog,, with defects randomly allocated to control or treatment group.	BMSCs collected from tibias	Autologous NA Passage 3	BMSC solution was 95% CD44+ and CD34-. BMSCs were labeled with BrdU and suspended into hydrogel (sodium alginate solution plus calcium chloride solution) Plaque control was achieved mechanically and by CHX for 6 weeks. Antibiotics were given for 3 days.	6 weeks None Split-mouth Yes, defects were randomly allocated.	Periodontal defect filled with hydrogel seeded with BMSCs Periodontal defect only without hydrogel	None	None Wound dehiscence (y/n) Infection (y/n)	Transplanted BMSCs differentiated into periodontal fibroblasts and osteoblasts, as well as vascular endothelial cells in the periodontal defect *in vivo*. BMSCs had the potential to regenerate periodontal tissue.	68.0%
Wei [97] **2012**	12 months NR Miniature pigs China	Mechanical Three-wall infra-bony defects Alveolar bone was removed using a surgical burr to create defects in the mesial region of the maxillary and mandibular first molars (3mm width, 7 mm length, and 5 mm depth).	Nine animals were used and two defects were generated per animal.	PDLSCs were obtained from minipig canine teeth	Autologous Enzymatic digestion Passage 3 to 4	Parts of the cell sheets were obtained from confluent cells on temperature-responsive culture dishes (UpCell dish). Another part of the cell sheets was obtained using vitamin C supplement until confluence. Not reported.	12 weeks None Split-mouth Yes, defects were randomly assigned to groups	Vitamin C-induced PDLSC sheets PDLSC sheets harvested from a temperature-responsive dish Dissociated PDLSCs combined with absorbable gelatin sponge None	Percentage of periodontal bone (%)	None None	PDLSC sheets were able to regenerate periodontal tissues. Vitamin C stimulation could be used to optimize this therapeutic approach.	34.5%
Zhao [98] **2004**	NR NR Athymic rat Brazil	Mechanical Fenestration defects Surgical operations were performed bilaterally on the mandible. Bone overlying the mandibular first molar was removed. Distal roots were	Twelve rats were used, 8 for 3 weeks and 4 for 6 weeks. 2 defects were created per rat. Defects were randomly allocated to one of the 3 cell	Primary follicle cells were isolated from the first molars of CD-1 mice.	Xenogeneic Enzymatic digestion Passage 2	PLGA was processed into porous foams by an established solvent casting. Scaffolds contained 95% porosity and pore sizes ranged from 250 to 425µm. Ampicillin was given for 7 days.	6 weeks 3 and 6 weeks Split-mouth Yes. Moreover, there was a single masked calibrated examiner.	Carrier with follicle cells Carrier alone Carrier with immortalized cementoblasts	Length of new bone bridge at 3 weeks (mm) Area of supporting newly formed mineralized tissue at 3 weeks (mm2) Area of total newly formed mineralized tissue at 3 weeks	None Neoplastic changes	Immortalized cementoblasts have the ability to repair periodontal defects by forming mineralized tissues *in vivo*; whereas follicle cells inhibit periodontal healing.	62.1%

		denuded of periodontal ligament, cementum and superficial dentine.	therapies, with the same treatment never being performed twice on the same rat.						(mm2) Defects fill at 3 weeks (%)			

Table S4A: Subgroup analyses for bone regeneration

Subgroups	Studies	Groups	References	SMD 95% CI	I^2	P
Bone regeneration (total)	24	35		0.66 [0.37, 0.95]	40%	***
Periodontal defects				Subgroup differences	P = 0.77	
Fenestration defects	9	13	[61, 62, 70, 72, 78, 82, 91, 93, 98]	0.51 [0.10, 0.92]	46%	**
Infra-bony defects	7	11	[7, 73, 76, 77, 80, 84, 95]	0.69 [0.34, 1.04]	60%	***
Furcation defects	9	11	[64, 65, 71, 74, 75, 77, 86-88, 90]	0.68 [0.30, 1.07]	0%	***
Defect generation				Subgroup differences	P = 0.85	
Mechanical	16	21	[61, 62, 64, 65, 70, 72, 73, 75, 78, 82, 87, 88, 90, 91, 93, 95, 98]	0.69 [0.28, 1.10]	42%	***
Inflammatory	8	14	[7, 71, 74, 76, 77, 80, 84, 86]	0.63 [0.20, 1.06]	41%	**
Gender				Subgroup differences	P = 0.74	
Male	8	14	[7, 62, 73, 76, 78, 84, 91, 93]	0.65 [0.30, 1.00]	0%	***
Female	6	10	[61, 70, 71, 75, 77, 82]	0.48 [0.02, 0.94]	24%	*
NR	10	11	[64, 65, 72, 74, 80, 86-88, 90, 95, 98]	0.80 [0.04, 1.55]	68%	*
Cell source				Subgroup differences	P = 0.71	
From dental tissues	14	16	[61, 64, 65, 70, 73, 76, 77, 80, 82, 84, 87, 88, 91, 95, 98]	0.63 [0.14, 1.12]	52%	***
From gingival tissues	2	3	[7, 95]	0.22 [-0.75, 1.20]	63%	NS
From oral bone tissues	3	4	[74, 77, 91]	0.89 [0.16, 1.62]	0%	*
From extra-oral bone marrow or adipose tissue	6	7	[62, 75, 86, 90, 91, 93]	0.94 [0.46, 1.41]	0%	***
Others	3	5	[71, 72, 78]	0.62 [-0.38, 1.62]	48%	NS
Animal model				Subgroup differences	P = 0.55	
Dog	13	16	[61, 64, 65, 73-76, 82, 84, 86-88, 90, 91]	0.62 [0.29, 0.94]	0%	***
Porcine	3	7	[7, 77, 80]	1.02 [0.36, 1.68]	52%	***
Rodent	6	8	[70, 72, 78, 93, 95, 98]	0.81 [-0.24, 1.85]	70%	NS
Graft				Subgroup differences	P = 0.04	
Autologous	17	25	[7, 61, 62, 64, 65, 73-77, 80, 82, 84, 86-88, 90, 91]	0.71 [0.45, 0.97]	4%	***
Allogeneic	3	3	[70, 78, 93]	1.86 [0.73, 2.98]	0%	***
Xenogeneic	5	7	[71, 72, 78, 95, 98]	0.07 [-0.77, 0.91]	64%	NS
Delivery				Subgroup differences	P = 0.18	
Bone substitute	7	9	[7, 73, 74, 76, 80, 86, 91]	0.81 [0.22, 1.39]	44%	**

PRP, EMD or blood clot	4	4	[64, 65, 71, 86, 90]	0.20 [-0.60, 1.00]	0%	NS
Cell sheets	4	6	[61, 71, 73, 91]	0.17 [-0.33, 0.67]	0%	NS
Collagen, gelatin, or acid hyaluronic	15	23	[7, 61, 70, 72, 75, 77, 78, 80, 82, 84, 87, 88, 91, 93, 95]	0.79 [0.43, 1.14]	39%	***
Polymers	5	8	[62, 71, 73, 91, 98]	0.20 [-0.50, 0.90]	51%	NS
Guided-tissue regeneration	10	16	[7, 61, 73, 74, 77, 80, 82, 87, 88, 91]	0.80 [0.42, 1.18]	30%	***
Randomization				Subgroup differences	P = 0.16	
Yes	13	18	[7, 62, 70, 71, 74, 76, 80, 82, 84, 87, 88, 95, 98]	0.46 [0.00, 0.91]	59%	*
No or not reported	10	17	[61, 64, 65, 72, 73, 75, 77, 78, 86, 90, 91]	0.87 [0.51, 1.22]	0%	***
Study duration				Subgroup differences	P = 0.71	
Six weeks and less than 6 weeks	6	4	[64, 65, 73, 75, 78, 82, 90]	0.67 [-0.13, 1.46]	34%	NS
Between 6 and 8 weeks	5	6	[61, 76, 86, 90, 91]	0.43 [-0.06, 0.92]	0%	NS
More than 8 weeks	8	16	[7, 71, 74, 77, 80, 84, 87, 88]	0.68 [0.31, 1.06]	32%	***
Quality of studies				Subgroup differences	P = 0.88	
High quality	7	10	[7, 70, 71, 80, 84, 86, 87]	0.68 [0.05, 1.32]	59%	*
Moderate quality	12	19	[61, 62, 73-77, 82, 88, 90, 91, 95]	0.60 [0.30, 0.90]	10%	***
Low quality	5	6	[64, 65, 72, 78, 93, 98]	0.93 [-0.47, 2.33]	65%	NS

Table 4SB: Subgroup analyses for cementum regeneration

				All studies		
Subgroups	**Studies**	**Groups**	**References**	**SMD 95% CI**	**I^2**	**P**
Cementum regeneration (total)	17	23		0.93 [0.62, 1.25]	19%	***
Periodontal defects				Subgroup differences	P = 0.49	
Fenestration defects	4	4	[61, 70, 82, 93]	0.55 [-0.10, 1.21]	0%	NS
Infra-bony defects	5	7	[7, 73, 76, 77, 84]	1.02 [0.56, 1.47]	0%	***
Furcation defects	9	11	[64, 65, 71, 74, 75, 77, 86-88, 90]	0.99 [0.42, 1.55]	43%	***
Defect generation				Subgroup differences	P = 0.14	
Mechanical	10	10	[61, 64, 65, 70, 73, 75, 82, 87, 88, 90, 93]	1.21 [0.69, 1.74]	25%	***
Inflammatory	7	13	[7, 71, 74, 76, 77, 84, 86]	0.74 [0.38, 1.10]	4%	***
Gender				Subgroup differences	P = 0.80	

Male	5	7	[7, 73, 76, 84, 93]	1.00 [0.47, 1.54]	16%	**
Female	6	10	[61, 70, 71, 75, 77, 82]	0.83 [0.28, 1.38]	44%	**
NR	6	6	[64, 65, 74, 86-88, 90]	1.10 [0.47, 1.73]	0%	***
Cell sources				Subgroup differences	P = 0.75	
From dental tissues	10	12	[61, 64, 65, 70, 73, 76, 77, 82, 84, 87, 88]	0.99 [0.58, 1.39]	0%	***
From oral bone tissues	2	3	[74, 77]	1.36 [0.47, 2.26]	0%	**
From extra-oral bone marrow or adipose tissue	4	4	[75, 86, 90, 93]	1.03 [-0.16, 2.23]	68%	NS
Animal model				Subgroup differences	P = 0.14	
Dog	12	13	[61, 64, 65, 73-76, 82, 84, 86-88, 90]	1.28 [0.89, 1.67]	0%	***
Porcine	2	6	[7, 77]	0.80 [0.28, 1.31]	4%	**
Delivery				Subgroup differences	P = 0.18	
Bone substitute	5	5	[7, 73, 74, 76, 86]	0.92 [0.35, 1.49]	0%	**
PRP, EMD or blood clot	4	4	[64, 65, 71, 86, 90]	0.57 [-0.25, 1.38]	0%	NS
Cell sheets	3	4	[61, 71, 73]	0.55 [-0.38, 1.48]	45%	NS
Collagen, gelatin, or acid hyaluronic	10	14	[7, 61, 70, 75, 77, 82, 84, 87, 88, 93]	1.12 [0.65, 1.59]	38%	***
Polymers	2	3	[71, 73]	0.69 [-0.70, 2.08]	63%	NS
Guided-tissue regeneration	8	12	[7, 61, 73, 74, 77, 82, 87, 88]	0.95 [0.58, 1.32]	0%	***
Randomization				Subgroup differences	P = 0.87	
Yes	9	12	[7, 70, 71, 74, 76, 82, 84, 87, 88]	0.91 [0.55, 1.27]	0%	***
No or not reported	8	11	[61, 64, 65, 73, 75, 77, 86, 90, 93]	0.97 [0.35, 1.60]	47%	***
Study duration				Subgroup differences	P = 0.60	
Six weeks and less	5	5	[64, 65, 73, 75, 82, 90]	1.18 [-0.04, 2.39]	63%	NS
Eight weeks and after	11	17	[7, 61, 71, 74, 76, 77, 84, 86-88, 90]	0.84 [0.53, 1.15]	0%	***

Legend: Standardized mean differences are presented with 95% confidence intervals; heterogeneity is represented by I^2 percentage. Significant subgroup differences are presented with P-values (significance level 0.1) whereas the significance for each subgroup is presented as follows: NS – not significant, * P<0.5, ** P<0.01, *** P<0.001. Abbreviations: EMD (Enamel Matrix Derivatives), NR (Not Reported), SMD (Standardized Mean Difference), PRP (Plasma Rich Platelet).

Synthesis: Cementum regeneration was greater in infra-bony defects (1.02 [0.56, 1.47]) compared to those with fenestration (0.55 [-0.10, 1.21]) and furcation defects (0.99 [0.42, 1.55]), and in defects

generated with mechanical process (1.21 [0.69, 1.74]) compared to defects generated with inflammatory process (0.74 [0.38, 1.10]). These subgroup differences were not statistically significant and such differences were not observed for bone regeneration. The gender of the animals did not affect the efficiency of cell grafting for cementum regeneration. Nevertheless, unknown sex (0.80 [0.04, 1.55]) and male (0.65 [0.30, 1.00]) groups displayed greater, but not statistically significant, bone regeneration compared to female groups (0.48 [0.02, 0.94]). Cell therapy groups (from dental tissues, oral bone tissues and extra-oral bone marrow or adipose tissue) displayed statistically greater bone and cementum regeneration ,compared to control groups, except for cementum regeneration by BMSCs or ADSCs (1.03 [-0.16, 2.23]). Autologous (0.71 [0.45, 0.97]) and allogeneic grafts (1.86 [0.73, 2.98]) produced significant more bone regeneration than xenogeneic grafting (0.07 [-0.77, 0.91]) (P=0.04). Bone and cementum regeneration were statistically greater compared to controls when cells were administered with bone substitute (respectively 0.81 [0.22, 1.39] and 0.92 [0.35, 1.49]) or with collagen, fibrin, hydrogel, gelatin, or hyaluronic acid carriers (0.79 [0.43, 1.14] and 1.12 [0.65, 1.59]) than when administered with PRP, EMD, blood clots, cell sheets or polymers. In large animals, bone regeneration using cells was found to be significantly greater than controls only when evaluated after 8 weeks (0.68 [0.31, 1.06]), compared to studies with shorter follow-up periods (less than 6 weeks (0.67 [-0.13, 1.46]) or between 6 and 8 weeks (0.43 [-0.06, 0.92]). Same results were obtained for cementum regeneration.

Table S5: Characteristics of included human studies

	Participants	Periodontal defects						Groups	Outcomes		Conclusion
	Age *Gender* *Country*	*Type of defects* *Description of defects*	*Number of defects*	*Type of cells*	*Type of graft* *Passage*	*Carrier*	*Total duration* *Timepoints* *Study design* *Randomization*	*Experimental groups* *Control groups*	*Clinical, radiological or histological parameters*	*Biological parameters* *Societal and patient outcomes*	*Main conclusion according study author*
Feng[99] 1992	25 to 46 years 4 males and 3 females Taiwan	Periodontal defects NR	Three defects in 3 patients for PDLSC group, 1 defect in 1 patient for gingival cells and 3 defects for 3 control patients (7 subjects)	PDLSCs were cultured from periodontal ligament of impacted third molars. Fibroblast-like cells were also obtained from interproximal gingiva	Autologous Explants method Passage 2 to 4	Cells were grown onto hydroxyapatite particles for 10-14 days	6 months 1, 3 and 6 months Case report Defects were randomized	PDLSCs, hydroxyapatite coated Gingival cells, hydroxyapatite coated Hydroxyapatite particles alone	Probing depth reduction (mm) Gingival recession (mm) Attachment gain (mm) Tooth mobility	None None	Application of periodontal ligament cells could facilitate earlier repopulation of root surface and improve clinical attachment level compared to control.
Feng[100] 1995	25 years Male Taiwan	Chronic periodontitis Deep pockets and vertical bony defect on the right maxillary first molar (mobility grade I, class II furcation lesion on buccal side, class I on mesial side)	One defect in one patient	Connective tissue was obtained from papilla	Autologous Explants method Passage 1	Hydroxyapatite particles were added in culture to be coated by cells	28 months 9 and 28 months Case report No	With hydroxyapatite and cells None	PPD (mm) CAL (mm) Gingival recession (mm) Furcation invasion (y/n) Tooth mobility (I, II, III)	None Post-operative complications (y/n)	Gingival fibroblast-like cells coating hydroxyapatite may lead to clinical improvement (pocket depth reduction, inflammation reduction) in periodontitis-affected osseous defects.
Feng[101] 2010	25, 25 and 42 years Male Taiwan	Chronic generalized periodontitis At least one deep infra-bony defect of ≥ 6mm.	Patients 1, 2 and 3 had respectively 12, 1 and 3 teeth for treatment, with some teeth showing furcation area involvement.	PDLSCs obtained from third molars	Autologous Explants method Passage 1	Bone grafting material CALCITITE 4060-2 was added to PDLSC culture at day 3 at passage 1.	32 to 72 months 3, 6, 12, 26, 32, 42 and 72 months Case report No	Periodontal defect was filled with PDLSCs and CALCITITE None	PPD and changes in PPD (mm) CAL and changes in CAL (mm) Recession and changes in recession (mm) Tooth mobility (0 to IV)	None None	This study provided clinical data supporting potential efficacy and safety of using autologous PDLSCs for human periodontal regeneration.
Hou[102] 2003	25, 29 years and NR Male, female and NR Taiwan	Periodontal defects Granulation tissues were removed and root planing was performed before graft implantation. Teeth treated included deep pockets and angular bony defects, class II and class I furcation lesions, tooth mobility and suppuration.	Four teeth in total on 3 patients were involved.	Gingival fibroblast-like cells were derived from the tissue culture of healthy buccal papillae between adjacent bicuspids.	Autologous Explants method Passage 1	Cells were co-cultured with hydroxyapatite.	72 months 3, 6, 9 and 36 months Case report No	Gingival fibroblast-like cells with hydroxyapatite Hydroxyapatite alone	Probing depth (mm) Furcation invasion (y/n) Tooth mobility (y/n) Recession (mm) Attachment gain (mm) Cemento-enamel junction to osseous defect (mm) Bottom of osseous defect to bone crest (mm) Furcation invasion (mm) Sign of ankylosis (y/n)	None None	Implantation of HA-cultured gingival fibroblast-like cells into periodontal defects was tolerated and was able to help periodontal tissue formation.
Mizuno [103] 2010	49, 41, 54 and 61 years 3 females and 1 male Japan	Chronic periodontitis Periodontitis was defined with PPD superior or equal to 6mm and CAL superior or equal to 4 mm at multiple sites.	One defect of 6 mm for patient 1, 2 defects of 6 mm for patient 2, 2 defects of 9 and 10 mm for patient 3 and 2 defects of 5 and 9 mm for patient 4.	Samples of periosteum were obtained from the mandibular body.	Autologous Explants method NR	Cells were incubated until sheet formation, about 4 weeks. Culture medium was supplemented with ascorbic acid. Two patients received platelet-rich plasma mixed with thrombin/calcium chloride. Cell sheet was placed around the exposed roots	12 to 14 months None Case report No	With cell sheet None	PPD (mm) Tooth mobility (y/n) Tooth mobility change (y/n)	None Post-operative complications (y/n)	Probing depths were reduced to normal depth and remained so beyond one year. Periosteal cells could be interesting for periodontal regeneration.
Okuda [104] 2009	71, 53 and 63 years 3 females Japan	Advanced chronic periodontitis Presence of one infra-bony defect with a PPD ≥ 6 mm, CAL ≥ 6 mm and osseous defect depth estimated to be ≥ 3 mm as measured radiographically.	One defect for patient 1, 2 defects for patients 2 and 3.	Periosteum samples were harvested from the mandible of each patient.	Autologous Explants method Passage 1	Cells were incubated until sheet formation, about 6 weeks. A coagulated preparation of PRPs was obtained by combining it with sodium alginate. Then HA granules were mixed with the PRPs before	6 months None Case report No	Cell sheet with PRP and HA None	PPD (mm) CAL (mm) Radiographic infra-bony defect depth (mm)	None Post-operative complications (y/n)	Periosteum cell sheets in combination with PRPs and HA granules showed favorable clinical improvement for infra-bony defects.

						being implanted into defects. Cell sheet was used to cover defect.					
Yamada [105] 2006	54 years Woman Japan	Infra-bony periodontal defect Granulation tissue from periodontal pocket was excised, root was scaled and planed. Stem cells were applied onto root.	One patient, one defect on tooth 45 (mean PPD of 4.50mm) and one defect on tooth 35 (mean PPD of 4.50mm)	BMSCs were isolated from patient iliac crest marrow aspirate. Cells were then cultured in osteogenic medium.	Autologous NA NR	BMSCs were resuspended in PRPs, and then mixed with thrombin-calcium chloride to form an insoluble gel.	1 year None Case report No	Regeneration on tooth 45 Regeneration on tooth 35	PPD (mm) CAL (mm)	None None	This study suggests that MSC/PRP gel could be clinically effective in reducing probing depth, improving attachment level in infrabony lesions.

Abbreviations: BMSCs – Bone Marrow Stromal Cells, CAL – Clinical Attachment Level, HA – Hydroxyapatite, PDLSCs – Periodontal Ligament Stromal Cells, PPD – Periodontal Pocket Depth, PRPs – Plasma Rich Platelets

In addition, there were 4 clinical trials identified from searching the grey literature. There were 2 uncontrolled trials, with 1 of them [106] aimed to inquire about the safety of autologous ADSCs for periodontal regeneration, but this study was not yet recruiting. In the other [107], the focus was on the safety and efficacy of a mixture containing MSCs, osteoblast-like cells differentiated from MSCs, PRP, thrombin and calcium chloride for the treatment of adult periodontitis patients. There was 1 randomized controlled trial [108] which investigated the safety and efficacy of PDLSC use for the regeneration of deep periodontal infrabony defects in 35 patients (this study was recruiting patients). In a pilot phase I clinical trial of 20 healthy male volunteers, no adverse reactions occurred with autologous periodontal ligament cells (PDLSCs) [108]. Indeed, 1 non-randomized clinical trial [109] on 80 patients with chronic periodontitis was aimed to compare safety and efficacy of periodontal regeneration by PDLSCs (prepared as cell sheets or pellets with bone substitute material) versus the standard of care or versus bone substitute alone.

Supplementary references

1. World Health Organisation International Clinical Trials Registry Platform. Available at www.who.int/trialsearch. Accessed August 28, 2013.

2. EUROPERIO meetings. Available at http://onlinelibrary.wiley.com/journal/10.1111/%28ISSN%291600-051X. Accessed August 28, 2013.

3. International Association for Dental Research meetings. Available at http://iadr.confex.com/iadr/search.epl. Accessed August 28, 2013.

4. Engel GL. The clinical application of the biopsychosocial model. **Am J Psychiatry**. 1980;137:535-544.

5. Vignoletti F, Abrahamsson I. Quality of reporting of experimental research in implant dentistry. Critical aspects in design, outcome assessment and model validation. **J Clin Periodontol**. 2012;39 Suppl 12:6-27.

6. Kilkenny C, Browne WJ, Cuthill IC et al. Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research. **J Pharmacol Pharmacother**. 2010;1:94-99.

7. Fawzy El-Sayed KM, Paris S, Becker ST et al. Periodontal regeneration employing gingival margin-derived stem/progenitor cells: an animal study. **J Clin Periodontol**. 2012;39:861-870.

8. Ding G, Liu Y, Wang W et al. Allogeneic periodontal ligament stem cell therapy for periodontitis in swine. **Stem Cells**. 2010;28:1829-1838.

9. Park JY, Jeon SH, Choung PH. Efficacy of periodontal stem cell transplantation in the treatment of advanced periodontitis. **Cell Transplant**. 2011;20:271-285.

10. Duan X, Tu Q, Zhang J et al. Application of induced pluripotent stem (iPS) cells in periodontal tissue regeneration. **J Cell Physiol**. 2011;226:150-157.

11. Li H, Yan F, Lei L et al. Application of autologous cryopreserved bone marrow mesenchymal stem cells for periodontal regeneration in dogs. **Cells Tissues Organs**. 2009;190:94-101.

12. Akbay A, Baran C, Gunhan O et al. Periodontal regenerative potential of autogenous periodontal ligament grafts in Class II furcation defects. **J Periodontol**. 2005;76:595-604.

13. Block MS. Use of living cell construct to enhance bone reconstruction: preliminary results. **J Oral Maxillofac Surg**. 2010;68:2914-2919.

14. Pérez Borrego A, Domínguez Rodríguez L, Ilisástigui Ortueta ZT et al. Utilización de células madre en el tratamiento de defectos óseos periodontales / Stem-cells used in treatment of periodontal bone defects. **Rev cuba estomatol**. 2009;46.

15. Pérez Borrego A, Ilisástigui Ortueta ZT, Hernández Ramírez P et al. Historia de la aplicación de la terapia celular en periodoncia. **Revista Habanera de Ciencias Médicas**. 2009;8:4-8.

16. Duailibi SE, Duailibi MT, Zhang W et al. Bioengineered dental tissues grown in the rat jaw. **J Dent Res**. 2008;87:745-750.

17. Flores MG, Hasegawa M, Yamato M et al. Cementum-periodontal ligament complex regeneration using the cell sheet technique. **J Periodontal Res**. 2008;43:364-371.

18. Han C, Yang Z, Zhou W et al. Periapical follicle stem cell: a promising candidate for cementum/periodontal ligament regeneration and bio-root engineering. **Stem Cells Dev**. 2010;19:1405-1415.

19. Hasegawa N, Kawaguchi H, Hirachi A et al. Behavior of transplanted bone marrow-derived mesenchymal stem cells in periodontal defects. **J Periodontol**. 2006;77:1003-1007.

20. Inoue T, Osada H, Shiigai T et al. An experimental study of osteogenesis by autografted dental pulp, periodontal ligament, and bone marrow in vivo. **Bull Tokyo Dent Coll**. 1993;34:183-190.

21. Ji K, Liu Y, Lu W et al. Periodontal tissue engineering with stem cells from the periodontal ligament of human retained deciduous teeth. **J Periodontal Res**. 2013;48:105-116.

22. Katayama A, Ota M, Sugito H et al. Effect of proliferating tissue on transplanted teeth in dogs. **Oral Surg Oral Med Oral Pathol Oral Radiol Endod**. 2006;101:e110-118.

23. Kawaguchi H, Hayashi H, Mizuno N et al. [Cell transplantation for periodontal diseases. A novel periodontal tissue regenerative therapy using bone marrow mesenchymal stem cells]. **Clin Calcium**. 2005;15:99-104.

24. Kawaguchi H, Kurihara H. [Clinical trial of periodontal tissue regeneration]. **Nihon Rinsho**. 2008;66:948-954.

25. Kim K, Lee CH, Kim BK et al. Anatomically Shaped Tooth and Periodontal Regeneration by Cell Homing. **Journal of dental research**. 2010;89:842-847.

26. Langenbach F, Naujoks C, Smeets R et al. Scaffold-free microtissues: differences from monolayer cultures and their potential in bone tissue engineering. **Clin Oral Investig**. 2013;17:9-17.

27. Li HX, Yan FH, Lei L et al. [Effect of cryopreservation of bone marrow stromal cells on periodontal tissue regeneration]. **Zhonghua Kou Qiang Yi Xue Za Zhi**. 2008;43:246-247.

28. Li YF, Yan FH, Zhong Q et al. [Effect of hBMP-7 gene modified bone marrow stromal cells on periodontal tissue regeneration]. **Zhonghua Yi Xue Za Zhi**. 2010;90:1427-1430.

29. Liu HC, E LL, Wang DS et al. Reconstruction of alveolar bone defects using bone morphogenetic protein 2 mediated rabbit dental pulp stem cells seeded on nano-hydroxyapatite/collagen/poly(L-lactide). **Tissue Eng Part A**. 2011;17:2417-2433.

30. Lu H, Wu ZF, Tian Y. [A study on the effects of cells and scaffolds tissue engineering on the periodontal regeneration]. **Zhonghua Kou Qiang Yi Xue Za Zhi**. 2004;39:189-192.

31. Ma Z, Li S, Song Y et al. The biological effect of dentin noncollagenous proteins (DNCPs) on the human periodontal ligament stem cells (HPDLSCs) in vitro and in vivo. **Tissue Eng Part A**. 2008;14:2059-2068.

32. Marei MK, Nouh SR, Saad MM et al. Preservation and regeneration of alveolar bone by tissue-engineered implants. **Tissue Eng**. 2005;11:751-767.

33. Marei MK, Saad MM, El-Ashwah AM et al. Experimental formation of periodontal structure around titanium implants utilizing bone marrow mesenchymal stem cells: a pilot study. **J Oral Implantol**. 2009;35:106-129.

34. McAllister BS. Stem cell-containing allograft matrix enhances periodontal regeneration: case presentations. **Int J Periodontics Restorative Dent**. 2011;31:149-155.

35. Modino SA, Sharpe PT. Tissue engineering of teeth using adult stem cells. **Arch Oral Biol**. 2005;50:255-258.

36. Murano Y, Ota M, Katayama A et al. Periodontal regeneration following transplantation of proliferating tissue derived from periodontal ligament into class III furcation defects in dogs. **Biomed Res**. 2006;27:139-147.

37. Neamat A, Gawish A, Gamal-Eldeen AM. beta-Tricalcium phosphate promotes cell proliferation, osteogenesis and bone regeneration in intrabony defects in dogs. **Arch Oral Biol**. 2009;54:1083-1090.

38. Tooth root and periodontal ligaments regenerated in animal model. **J Am Dent Assoc**. 2007;138:158-160.

39. Ou L, Liu H, Pang J. [Effects of autogenous periodontal ligament cell transplantation on periodontal tissue regeneration in dogs]. **Zhonghua Kou Qiang Yi Xue Za Zhi**. 2000;35:44-46.

40. Ou L, Liu H, Wang D et al. [The study of calcification of autogenous bone marrow stem cell transplantation on alveolar bone defect in dogs]. **Zhonghua Kou Qiang Yi Xue Za Zhi**. 2002;37:132-134.

41. Reichert da Silva Assuncao L, Colenci R, Ferreira do-Amaral CC et al. Periodontal tissue engineering after tooth replantation. **J Periodontol**. 2011;82:758-766.

42. Ribeiro FV, Suaid FF, Ruiz KG et al. Peri-implant reconstruction using autologous periosteum-derived cells and guided bone regeneration. **J Clin Periodontol**. 2010;37:1128-1136.

43. Ribeiro FV, Suaid FF, Ruiz KG et al. Effect of autologous bone marrow-derived cells associated with guided bone regeneration or not in the treatment of peri-implant defects. **Int J Oral Maxillofac Surg**. 2012;41:121-127.

44. Seo BM, Miura M, Gronthos S et al. Investigation of multipotent postnatal stem cells from human periodontal ligament. **Lancet**. 2004;364:149-155.

45. Song H, Li Q, Li B et al. [Enhancing effect of tissue engineered bone on bone defect repair in rats]. **Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi**. 2009;23:1250-1253.

46. Song JS, Kim SO, Kim SH et al. In vitro and in vivo characteristics of stem cells derived from the periodontal ligament of human deciduous and permanent teeth. **Tissue Eng Part A**. 2012;18:2040-2051.

47. Takedachi M, Sawada K, Yamamoto S et al. Periodontal tissue regeneration by transplantation of adipose tissue-derived stem cells. **Journal of Oral Biosciences**. 2013;55:137-142.

48. Tobita M, Uysal AC, Ogawa R et al. Periodontal tissue regeneration with adipose-derived stem cells. **Tissue Eng Part A**. 2008;14:945-953.

49. Uematsu K, Kawase T, Nagata M et al. Tissue culture of human alveolar periosteal sheets using a stem-cell culture medium (MesenPRO-RS): In vitro expansion of CD146-positive cells and concomitant upregulation of osteogenic potential in vivo. **Stem Cell Res**. 2013;10:1-19.

50. Xu CJ, Guo F, Gao QP et al. [Effects of astragalus polysaccharides-chitosan/polylactic acid scaffolds and bone marrow stem cells on repairing supra-alveolar periodontal defects in dogs]. **Zhong Nan Da Xue Xue Bao Yi Xue Ban**. 2006;31:512-517.

51. Yang ZH, Jin F, Zhang XJ et al. A novel possible strategy based on self-assembly approach to achieve complete periodontal regeneration. **Artif Organs**. 2010;34:603-609.

52. Yang B, Chen G, Li J et al. Tooth root regeneration using dental follicle cell sheets in combination with a dentin matrix - based scaffold. **Biomaterials**. 2012;33:2449-2461.

53. Yokoi T, Saito M, Kiyono T et al. Establishment of immortalized dental follicle cells for generating periodontal ligament in vivo. **Cell Tissue Res**. 2007;327:301-311.

54. Yoshida T, Washio K, Iwata T et al. Current status and future development of cell transplantation therapy for periodontal tissue regeneration. **Int J Dent**. 2012;2012:307024.

55. Young CS, Terada S, Vacanti JP et al. Tissue engineering of complex tooth structures on biodegradable polymer scaffolds. **J Dent Res**. 2002;81:695-700.

56. Young CS, Kim SW, Qin C et al. Developmental analysis and computer modelling of bioengineered teeth. **Arch Oral Biol**. 2005;50:259-265.

57. Young CS, Abukawa H, Asrican R et al. Tissue-engineered hybrid tooth and bone. **Tissue Eng**. 2005;11:1599-1610.

58. Zhang W, Abukawa H, Troulis MJ et al. Tissue engineered hybrid tooth-bone constructs. **Methods**. 2009;47:122-128.

59. Zhang L, Wang P, Mei S et al. In vivo alveolar bone regeneration by bone marrow stem cells/fibrin glue composition. **Arch Oral Biol**. 2012;57:238-244.

60. Zuolin J, Hong Q, Jiali T. Dental follicle cells combined with beta-tricalcium phosphate ceramic: a novel available therapeutic strategy to restore periodontal defects. **Med Hypotheses**. 2010;75:669-670.

61. Akizuki T, Oda S, Komaki M et al. Application of periodontal ligament cell sheet for periodontal regeneration: a pilot study in beagle dogs. **J Periodontal Res**. 2005;40:245-251.

62. Chen YL, Chen PK, Jeng LB et al. Periodontal regeneration using ex vivo autologous stem cells engineered to express the BMP-2 gene: an alternative to alveolaplasty. **Gene Ther**. 2008;15:1469-1477.

63. Chung VH, Chen AY, Kwan CC et al. Mandibular alveolar bony defect repair using bone morphogenetic protein 2-expressing autologous mesenchymal stem cells. **J Craniofac Surg**. 2011;22:450-454.

64. Dogan A, Ozdemir A, Kubar A et al. Assessment of periodontal healing by seeding of fibroblast-like cells derived from regenerated periodontal ligament in artificial furcation defects in a dog: a pilot study. **Tissue Eng**. 2002;8:273-282.

65. Dogan A, Ozdemir A, Kubar A et al. Healing of artificial fenestration defects by seeding of fibroblast-like cells derived from regenerated periodontal ligament in a dog: a preliminary study. **Tissue Eng**. 2003;9:1189-1196.

66. Flores MG, Yashiro R, Washio K et al. Periodontal ligament cell sheet promotes periodontal regeneration in athymic rats. **J Clin Periodontol**. 2008;35:1066-1072.

67. Grimm WD, Dannan A, Becher S et al. The ability of human periodontium-derived stem cells to regenerate periodontal tissues: a preliminary in vivo investigation. **Int J Periodontics Restorative Dent**. 2011;31:e94-e101.

68. Guo W, He Y, Tang X et al. Scaffold-free cell pellet transplantations can be applied to periodontal regeneration. **Cell Transplant**. 2013.

69. Hasegawa M, Yamato M, Kikuchi A et al. Human periodontal ligament cell sheets can regenerate periodontal ligament tissue in an athymic rat model. **Tissue Eng**. 2005;11:469-478.

70. Han J, Menicanin D, Marino V et al. Assessment of the regenerative potential of allogeneic periodontal ligament stem cells in a rodent periodontal defect model. **J Periodontal Res**. 2013.

71. Hovey LR, Jones AA, McGuire M et al. Application of periodontal tissue engineering using enamel matrix derivative and a human fibroblast-derived dermal substitute to stimulate periodontal wound healing in Class III furcation defects. **J Periodontol**. 2006;77:790-799.

72. Hynes K, Menicanin D, Han J et al. Mesenchymal Stem Cells from iPS Cells Facilitate Periodontal Regeneration. **J Dent Res**. 2013;92:833-839.

73. Iwata T, Yamato M, Tsuchioka H et al. Periodontal regeneration with multi-layered periodontal ligament-derived cell sheets in a canine model. **Biomaterials**. 2009;30:2716-2723.

74. Jiang J, Wu X, Lin M et al. Application of autologous periosteal cells for the regeneration of class III furcation defects in Beagle dogs. **Cytotechnology**. 2010;62:235-243.

75. Kawaguchi H, Hirachi A, Hasegawa N et al. Enhancement of periodontal tissue regeneration by transplantation of bone marrow mesenchymal stem cells. **J Periodontol**. 2004;75:1281-1287.

76. Khorsand A, Eslaminejad MB, Arabsolghar M et al. Autologous dental pulp stem cells in regeneration of defect created in canine periodontal tissue. **J Oral Implantol**. 2013;39:433-443.

77. Lang H, Schuler N, Nolden R. Attachment formation following replantation of cultured cells into periodontal defects--a study in minipigs. **J Dent Res**. 1998;77:393-405.

78. Lekic PC, Rajshankar D, Chen H et al. Transplantation of labeled periodontal ligament cells promotes regeneration of alveolar bone. **Anat Rec**. 2001;262:193-202.

79. Lekic PC, Nayak BN, Al-Sanea R et al. Cell transplantation in wounded mixed connective tissues. **Anat Rec A Discov Mol Cell Evol Biol**. 2005;287:1256-1263.

80. Liu Y, Zheng Y, Ding G et al. Periodontal ligament stem cell-mediated treatment for periodontitis in miniature swine. **Stem Cells**. 2008;26:1065-1073.

81. Mizuno H, Hata K, Kojima K et al. A novel approach to regenerating periodontal tissue by grafting autologous cultured periosteum. **Tissue Eng**. 2006;12:1227-1335.

82. Nakahara T, Nakamura T, Kobayashi E et al. In situ tissue engineering of periodontal tissues by seeding with periodontal ligament-derived cells. **Tissue Eng**. 2004;10:537-544.

83. Nayak BN, Wiltshire WA, Ganss B et al. Healing of periodontal tissues following transplantation of cells in a rat orthodontic tooth movement model. **Angle Orthod**. 2008;78:826-831.

84. Nunez J, Sanz-Blasco S, Vignoletti F et al. Periodontal regeneration following implantation of cementum and periodontal ligament-derived cells. **J Periodontal Res**. 2012;47:33-44.

85. Park CH, Rios HF, Jin Q et al. Tissue engineering bone-ligament complexes using fiber-guiding scaffolds. **Biomaterials**. 2012;33:137-145.

86. Simsek SB, Keles GC, Baris S et al. Comparison of mesenchymal stem cells and autogenous cortical bone graft in the treatment of class II furcation defects in dogs. **Clin Oral Investig**. 2012;16:251-258.

87. Suaid FF, Ribeiro FV, Rodrigues TL et al. Autologous periodontal ligament cells in the treatment of class II furcation defects: a study in dogs. **J Clin Periodontol**. 2011;38:491-498.

88. Suaid FF, Ribeiro FV, Gomes TR et al. Autologous periodontal ligament cells in the treatment of Class III furcation defects: a study in dogs. **J Clin Periodontol**. 2012;39:377-384.

89. Tan Z, Zhao Q, Gong P et al. Research on promoting periodontal regeneration with human basic fibroblast growth factor-modified bone marrow mesenchymal stromal cell gene therapy. **Cytotherapy**. 2009;11:317-325.

90. Tobita M, Uysal CA, Guo X et al. Periodontal tissue regeneration by combined implantation of adipose tissue-derived stem cells and platelet-rich plasma in a canine model. **Cytotherapy**. 2013.

91. Tsumanuma Y, Iwata T, Washio K et al. Comparison of different tissue-derived stem cell sheets for periodontal regeneration in a canine 1-wall defect model. **Biomaterials**. 2011;32:5819-5825.

92. van Dijk LJ, Schakenraad JM, van der Voort HM et al. Cell-seeding of periodontal ligament fibroblasts. A novel technique to create new attachment. A pilot study. **J Clin Periodontol**. 1991;18:196-199.

93. Yang Y, Rossi FM, Putnins EE. Periodontal regeneration using engineered bone marrow mesenchymal stromal cells. **Biomaterials**. 2010;31:8574-8582.

94. Yang JR, Hsu CW, Liao SC et al. Transplantation of embryonic stem cells improves the regeneration of periodontal furcation defects in a porcine model. **J Clin Periodontol**. 2013.

95. Yu N, Oortgiesen DA, Bronckers AL et al. Enhanced periodontal tissue regeneration by periodontal cell implantation. **J Clin Periodontol**. 2013;40:698-706.

96. Wei N, Gong P, Liao D et al. Auto-transplanted mesenchymal stromal cell fate in periodontal tissue of beagle dogs. **Cytotherapy**. 2010;12:514-521.

97. Wei F, Qu C, Song T et al. Vitamin C treatment promotes mesenchymal stem cell sheet formation and tissue regeneration by elevating telomerase activity. **J Cell Physiol**. 2012;227:3216-3224.

98. Zhao M, Jin Q, Berry JE et al. Cementoblast delivery for periodontal tissue engineering. **J Periodontol**. 2004;75:154-161.

99. Feng F, Hou LT. Treatment of osseous defects with fibroblast-coated hydroxylapatite particles. **J Formos Med Assoc**. 1992;91:1068-1074.

100. Feng F, Liu CM, Hsu WC et al. Long-term effects of gingival fibroblast-coated hydroxylapatite graft on periodontal osseous defects. **J Formos Med Assoc**. 1995;94:494-498.

101. Feng F, Akiyama K, Liu Y et al. Utility of PDL progenitors for in vivo tissue regeneration: a report of 3 cases. **Oral Dis**. 2010;16:20-28.

102. Hou LT, Tsai AY, Liu CM et al. Autologous transplantation of gingival fibroblast-like cells and a hydroxylapatite complex graft in the treatment of periodontal osseous defects: cell cultivation and long-term report of cases. **Cell Transplant**. 2003;12:787-797.

103. Mizuno H, Kagami H, Mase J et al. Efficacy of membranous cultured periosteum for the treatment of patients with severe periodontitis: a proof-of-concept study. **Nagoya J Med Sci**. 2010;72:59-70.

104. Okuda K, Yamamiya K, Kawase T et al. Treatment of human infrabony periodontal defects by grafting human cultured periosteum sheets combined with platelet-rich plasma and porous hydroxyapatite granules: case series. **J Int Acad Periodontol**. 2009;11:206-213.

105. Yamada Y, Ueda M, Hibi H et al. A novel approach to periodontal tissue regeneration with mesenchymal stem cells and platelet-rich plasma using tissue engineering technology: A clinical case report. **Int J Periodontics Restorative Dent**. 2006;26:363-369.

106. Kitamura M. An exploratory open trial to examine the safety of transplantation of autologous adipose tissue-derived stem cells for periodontal regeneration. Available at: http://apps.who.int/trialsearch/trial.aspx?trialid=JPRN-UMIN000007698.

107. Baba S. Clinical Trials of Regeneration for Periodontal Tissue. Available at: http://clinicaltrials.gov/ct2/show/NCT00221130.

108. Shi S. Periodontal Tissue Regeneration Using Autologous Periodontal Ligament Stem Cells (PDLSC). Available at: http://clinicaltrials.gov/ct2/show/NCT01357785.

109. Jin Y. Periodontal Ligament Stem Cell Implantation in the Treatment of Periodontitis. Available at: http://clinicaltrials.gov/ct2/show/NCT01082822.

frontiers in
PHYSIOLOGY

MINI REVIEW ARTICLE
published: 15 November 2013
doi: 10.3389/fphys.2013.00325

Cell therapy of periodontium: from animal to human?

Elena A. Trofin[1,2], Paul Monsarrat[1,3]* and Philippe Kémoun[1]

[1] *Department of Biology, Toulouse Faculty of Dentistry - Paul Sabatier University, Toulouse University Hospital - CHU de Toulouse, Toulouse, France*
[2] *Department of Pathology, Faculty of Medicine and Odontology, University of Valencia, Valencia, Spain*
[3] *Department of Public Health, Toulouse Faculty of Dentistry - Paul Sabatier University, Toulouse University Hospital - CHU de Toulouse, Toulouse, France*

Edited by:
Jean-Christophe Farges, University Lyon 1, France

Reviewed by:
Natalina Quarto, Università di Napoli Federico II, Italy
Javier Catón, Universidad Complutense de Madrid, Spain

****Correspondence:***
Paul Monsarrat, Département de Biologie, Faculté de chirurgie dentaire - Université Paul Sabatier 3, chemin des maraîchers, 31062 Cedex 09 Toulouse, France
e-mail: paul.monsarrat@gmail.com

Periodontitis is a chronic inflammatory disease affecting the soft and hard tissues supporting the teeth, which often leads to tooth loss. Its significant impact on the patient's general health and quality of life point to a need for more effective management of this condition. Existing treatments include scaling/root planning and surgical approaches but their overall effects are relatively modest and restricted in application. The goal of regenerative therapy of periodontal defects is to enhance endogenous progenitors and thus promote optimal wound healing. Considering that the host or tissue might be defective in the periodontitis context, it has been proposed that grafting exogenous stem cells would produce new tissues and create a suitable microenvironment for tissue regeneration. Thus, cell therapy of periodontium has been assessed in many animal models and promising results have been reported. However, the methodological diversity of these studies makes the conversion to clinical practice difficult. The aim of this review is to highlight the primary requirements to be satisfied before the leap to clinical trials can be made. We therefore review cell therapy applications for periodontal regeneration in animal models and the concerns to be addressed before undertaking human experiments.

Keywords: mesenchymal stromal cells, periodontitis, tissue engineering, bone regeneration, clinical trials as topic

INTRODUCTION

Periodontitis is an immuno-infectious disease, characterized by loss of both the soft and hard tissues anchoring the teeth. Left untreated, it leads to tooth loss (Pihlstrom et al., 2005). Chronic periodontitis is found in 15–50% of adults in developed countries (Oliver et al., 1998; Bourgeois et al., 2005). Conventional treatment, including oral hygiene instructions, and scaling/root planing, aims to prevent the disease, or slow or stop its progress, and maintain the therapeutic goals achieved but is usually insufficient to promote the regeneration of damaged structures (Bosshardt and Sculean, 2009). Deep infrabony defects associated with periodontal pockets are the classic indication for surgical periodontal regenerative therapy (Ramseier et al., 2012). Nevertheless, outcomes of existing procedures [guided tissue regeneration (GTR), Enamel-Matrix Derivatives (EMD) or Platelet Rich Plasma (PRP)], are not predictable and are associated with a relatively high degree of variability (Needleman et al., 2006; Esposito et al., 2009).

The regenerative mechanisms of mesenchymal stromal cell (MSC) grafts include direct commitment toward differentiating cells together with paracrine communication with resident connective cells, and infiltration of inflammatory cells, antigen-presenting cells, or both (Sorrell and Caplan, 2010). Paracrine interactions require these cells to produce and respond to a variety of trophic factors that may stimulate the resident cells to differentiate and themselves renew the pathological tissue (Baraniak and McDevitt, 2010). In addition, MSCs also exhibit immuno-modulatory functions, making them a potential tool to combat an immuno-infectious disease such as periodontitis. The reduction of inflammation by MSCs may halt the development of injury and allow regenerative processes to take place (Sensebe et al., 2010). Furthermore, MSCs may exert a neovascularization effect (Wu et al., 2007). Thus, one of the therapeutic functions of MSCs is the early induction of granulation tissue followed by the stabilization of the neovascular network in the periodontal niche (Sorrell and Caplan, 2010).

The graft of exogenous cells producing new tissues and/or making the local microenvironment suitable for an optimal activation of *in-situ* progenitors (Ilic and Polak, 2012; Shin and Peterson, 2013) has been tested in many models. Adult multipotent mesenchymal stromal cells, from bone marrow (BMSCs) or adipose tissue (adipose-derived stem cells or ASCs), are promising in the treatment of human diseases like graft-vs-host disease, ischemic cardiovascular diseases or large bone defects (Bourin et al., 2010; Sensebe et al., 2010). The main features of such cells include a capacity to self-renew and to undergo extensive proliferation and differentiation to various mesenchymal lineages (Dominici et al., 2006). Considering that the host or tissue might be defective in the periodontitis context, grafting exogenous MSCs that produce new tissues and create a suitable microenvironment for tissue regeneration has been proposed (Chen et al., 2012a). However, the methodological diversity of the available studies makes the conversion to clinical practice difficult. The aim of this review is, first, to report recent findings of periodontal regeneration in animal models and, second, to highlight the primary requirements to be satisfied before the leap to clinical trials can be made.

RESULTS FROM ANIMAL STUDIES

Advances in mesenchymal stem cell isolation, growth factor biology, and biodegradable polymer constructs have set the stage for successful tissue engineering of the periodontium, basically in animal models. Our screening of the literature revealed that data were available for about fifty studies. The majority of them concerned periodontal defects generated mechanically (burs) on dogs and rodents. Except for nude rodents where xenogeneic cells were used, most studies used autologous cell grafts. Periodontal ligament stromal cells (PDLSCs) were employed in about half the studies.

Table 1 outlines the general methodology from recent papers investigating periodontal regeneration of various defect models in a range of large animals. These studies were selected both for their methodological quality and to reflect the heterogeneity of methodologies used. We voluntarily did not select studies on rodents because of the low similarity between their physiopathology and that of humans, although they are considered as essential models for preliminary protocols before moving on to large-animal trials. Overall, the outcomes reported (**Table 2**) suggest that MSCs have the ability to enhance the regeneration of functional periodontal apparatus: newly formed bone and cementum with well-oriented ligament fibers.

RESULTS FROM HUMAN STUDIES

We identified 7 case reports (for a total of 22 patients) where MSCs had been applied clinically for infra-bony defects and furcation involvement after chronic periodontitis in humans. The efficacy of human periodontal cell therapy by grafting autologous stromal cells from gingiva or periodontal ligament with a hydroxyapatite (HA) carrier has been assessed since 1992 (Feng and Hou, 1992; Feng et al., 1995, 2010; Hou et al., 2003). Cultured mandibular periosteum-derived cell sheets with PRP have also been used in the treatment of chronic periodontitis without (Mizuno et al., 2010) or with HA in patients suffering from advanced chronic periodontitis (Okuda et al., 2009). A BMSC-PRP gel has also been used in an infrabony periodontal defect and led to a 4 mm clinical attachment gain (Yamada et al., 2006). Results suggest promising improvements in pocket depth, attachment gain and tooth mobility, compared to control groups when present. These data suggest MSCs may induce efficient and safe periodontal regeneration in humans.

Through the International Clinical Trials Registry Platform (World Health Organization), we identified four clinical studies (last access 2013/08/27): two single-arm studies using (i) ASCs for 12 patients with deep infra-bony defects (recruiting) and (ii) a mixture of *ex-vivo* cultured MSCs and *ex-vivo* cultured osteoblast-like cells differentiated from MSCs for 10 patients with chronic periodontitis (completed); and two trials investigating the safety and efficacy of PDLSCs in chronic periodontitis, one randomized and one non-randomized controlled trial, with respectively 35 and 80 patients (recruiting).

Nevertheless, results from case reports and clinical trials should be interpreted with caution. Although the clinical potential of MSCs in tissue regeneration appears to be established, the mechanisms involved in these processes after transplantation are not clearly understood (Chen et al., 2012b; Hoogduijn and Dor, 2013). Moreover, the clinical indications in periodontology remain be defined (Chen and Jin, 2010); the multiplicity of factors to be taken into account makes the regeneration equation more complex. Future studies should discuss regeneration according to the type of periodontal defects (number of walls), the type of periodontitis (chronic, aggressive) or the method used to generate the periodontitis model in animals, whether the MSCs are autologous, allogeneic or xenogeneic, the type of animal (dog, pig), the source tissue of MSCs (dental, oral, or extra-oral sources), the scaffold used, adjunctive growth factors or specific culture medium, etc.

KEY ELEMENTS TO CONSIDER BEFORE MAKING THE STEP TO CLINICAL TRIALS

The transfer of pre-clinical data to the clinical setting could be challenging and time consuming. So we will discuss some elements related to the choice of the origin of MSCs, their potential toxicity and their carrier, some laboratory considerations, the extent to which animal models reflect the applicability of the technique in humans, and regulatory information.

CELL SOURCES

One of the most important items of information that could help with the predictability of clinical results is the type of cell used. On the one hand, each tissue source has its own biological features and, even when they have common surface markers, these cells are determined by their original environment and may possibly be involved in specific differentiation pathways (Lin et al., 2009). On the other hand, the local microenvironment and surrounding tissue are important factors that influence the cell fate of whatever cells are ultimately used (Chen et al., 2011).

As stated above, PDLSCs are the cells most used for clinical trials, probably because of their periodontal origin and promising results in both animal and human trials (Feng et al., 2010; Suaid et al., 2012). Even if the choice of stromal cells from the oral niche (pulp, ligament, gingiva or oral alveolar bone) seems rational when the aim is to regenerate periodontal structures (Lin et al., 2009), periodontitis defects need a large number of cells (about 10^7 cells are needed for one defect), which would be impossible to obtain from a single subject. Thus, the respect of ethical considerations (e.g., putative removal of healthy teeth, increasing genetic instability through passages during cellular expansion) requires other sources of MSCs to be sought.

Two major locations of available MSCs are long-bone marrow and adipose tissue. These cells are morphologically and immunophenotypically similar to PDLSCs (Huang et al., 2009). Unlike BMSCs, ASCs can be recovered easily in large numbers by means of liposuction under local anesthesia. Indeed, adipose tissue is the richest source of MSCs, 100 times more than bone marrow (Bourin et al., 2010). Although ASCs may exhibit a reduced ability to differentiate into bone and cartilage (Kern et al., 2006) compared to BMSC or oral mesenchymal cells, they have given promising results in periodontal regeneration in dogs (Takedachi et al., 2013; Tobita et al., 2013).

The need for high cell concentrations requires the optimal type of transplant to be determined. To become a clinical reality, xenografts need to overcome immunological, physiological,

Table 1 | General methodology of cell therapy in animal subjects.

References	Animal	Type of defect	Cell type Type of graft	Carrier	Groups	Outcomes
Akizuki et al., *J. Periodontal. Res.*, 2005	Dog	Fenestration defects	PDLSCs Autologous	PDLSC single-layered sheets, reinforced using hyaluronic acid sheet and applied onto roots	PDLSC sheet with hyaluronic acid carrier Hyaluronic acid alone	Histomorphometric Post-operative complications
Chen et al., *Gene Ther.*, 2008	Rabbit	Fenestration defects Trans gingival periodontal defect	BMSCs transfected to over-express BMP-2 Autologous	Pluronic F127, solidified into gel form in incubator	BMP-2 gene-transfected BMSCs with PF127 Control transfected BMSCs with PF127 Untransfected BMSCs with PF127 PF127 only	Histologic 3D micro-CT Ankylosis Post-operative complications
Ding et al., *Stem Cells*, 2010	Minipig	Three-wall infra-bony defects	PDLSCs Autologous Allogeneic	PDLSCs cultured with HA/TCP to obtain cell sheets. Gelatin membranes covered defects	Autologous cells with HA/TCP Allogeneic cells with HA/TCP Autologous heterogenic with HA/TCP HA/TCP only No carrier	Clinical assessments Histomorphometric Immune assays Rejection
Fawzy El-Sayed et al., *J. Clin. Periodontol.*, 2012	Minipig	Two-wall infra-bony defects Silk sutures around cervical region of teeth were used to induce inflammation	Gingival margin-derived progenitor cells Autologous	Inorganic: deproteinized bovine cancellous bone (DBCB) Organic: collagen scaffold A collagen membrane was added to cover defects	Cells with DBCB Cells with collagen scaffold DBCB only Collagen scaffold only No carrier	Clinical assessments Radiographic Histomorphometric Ankylosis Root resorption
Hasegawa et al., *J. Periodontol.*, 2006	Dog	Class III furcation defect	BMSCs Autologous	Atelocollagen	BMSCs in atelocollagen Atelocollagen only	Histologic Immunohistochemistry
Iwata et al., *Biomaterials*, 2009	Dog	Three-wall infra-bony defect	PDLSCs Culture in an osteogenic medium Autologous	Tri-layered cell sheets formed with PDLSCs and sheets of polyglycolic acid (PGA). Defects were also filled with βTCP	Cell sheets with βTCP PGA sheets only with βTCP	Histomorphometric Ankylosis Post-operative complications
Li et al., *Cells Tissues Organs*, 2009	Dog	Fenestration defects	BMSCs, cryopreserved or not	Collagen membranes serving as carrier and applied onto roots. e-PTFE membranes covered defects	Cryopreserved BMSCs Non-cryopreserved BMSCs No cells	Histomorphometric Post-operative complications

(Continued)

Table 1 | Continued

References	Animal	Type of defect	Cell type Type of graft	Carrier	Groups	Outcomes
Liu et al., *Stem Cells*, 2008	Minipig	Two-wall infra-bony defects Silk sutures around cervical region of teeth were used to induce inflammation	PDLSCs	PDLSCs combined with HA/TCP with a gelatin membrane covering	PDLSCs with HA/TCP HA/TCP only No treatment	Clinical observations Histomorphometric Radiographic
Nunez et al., *J. Periodontal. Res.*, 2012	Dog	Three-wall infra-bony defects	Cementum and periodontal ligament-derived cells (CDCs and PDLSCs) Autologous	Collagen sponge	Collagen sponge with PDLDCs Collagen sponge with CDCs Collagen sponge with culture medium (control)	Histomorphometric Clinical measurements Post-operative complications Ankylosis Root resorption
Park et al., *Cell Transplant.*, 2011	Dog	Fenestration defects: apical involvement defects	Dental pulp, periodontal ligament and peri-apical follicular stem cells Autologous	None	PDLSC graft group Dental pulp stem cell graft group Peri-apical follicular stem cell graft group No surgical intervention Periodontal defect without cells	Clinical measurements 3D micro-CT Histologic Post-operative complications Neoplasm formation
Simsek et al., *Clin. Oral Investig.*, 2012	Dog	Class II furcation defect Defects were filled with rubber impression paste to induce inflammation	BMSCs Autologous	PRP mixed with BMSCs and autogenous cortical bone (ACB) also added into defects	BMSCs with PRP and ACB PRP and ACB PRP alone ACB alone No carrier (scaling and root planning only)	Clinical observations Histomorphometric Post-operative complications Root resorption Ankylosis
Suaid et al., *J. Clin. Periodontol.*, 2012	Dog	Class III furcation defect	PDLSCs Autologous	Collagen sponges were seeded with cells and membranes were used for guided tissue regeneration	Collagen sponge with cells Collagen sponge without cells Collagen sponge only Guided tissue regeneration only Surgical act only	Clinical observations Histomorphometric Post-operative complications Root resorption Ankylosis
Suaid et al., *J. Clin. Periodontol.*, 2011	Dog	Class II furcation defect	PDLSCs Autologous	Collagen sponges were seeded with cells and membranes were used for guided tissue regeneration	Collagen sponge with cells Collagen sponge without cells	Clinical observations Histomorphometric Post-operative complications Ankylosis

(Continued)

Table 1 | Continued

References	Animal	Type of defect	Cell type	Carrier	Groups	Outcomes
			Type of graft			
Takedachi et al., *J. Oral Biosci.*, 2013	Dog	Two-wall and furcation class II defects	ASCs Autologous	Fibrin gel	ASCs mixed with fibrin gel Fibrin gel alone	Radiographic 3D micro-CT Histologic Post-operative complications
Tobita et al., *Cytotherapy*, 2013	Dog	Class III furcation defect	ASCs Autologous	Autologous PRP was prepared to be mixed with cells	ASC seeded in PRP PRP alone No implantation	Radiographic Histomorphometric Immunohistochemistry
Tsumanuma et al., *Biomaterials*, 2011	Dog	One-wall infra-bony defect	Alveolar periosteum-derived stromal cells, PDLSCs and BMSCs Autologous	Tri-layered cell sheets were constructed using PGA, defects were also filled with β-TCP and type I collagen	Cell sheets with BMSCs Cell sheets with PDLSCs Cell sheets with APCs PGA sheet without cells	Clinical observations Histomorphometric Immunohistochemistry Post-operative complications Ankylosis Root resorption

Abbreviations: ACB, autogenous cortical bone; ASC, adipose-derived stem cell; BMP2, bone morphogenetic protein-2; BMSC, bone marrow stromal cell; DBCB, deproteinized bovine cancellous bone; CDC, cementum derived cell; e-PTFE, expanding polytetrafluoroethylene; HA, hydroxyapatite; PDLSC, periodontal ligament stromal cell; PF127, Pluronic F127; PGA, polyglycolic acid; TCP, tricalcium phosphate.

and infectious obstacles (Poncelet et al., 2009). For example, the donor might be genetically modified to protect its cells from the human immune system (Li et al., 2012). MSCs are both immunosuppressive and immunoprivileged and, as such, may be used as an allogeneic source of cells. A recent randomized controlled trial showed significant benefits and low rates of immunologic reactions for transendocardial injection of allogenic BMSCs in patients with ischemic cardiomyopathy (Hare et al., 2012). Another limitation is the time required to produce the MSCs. Cell storage may be used to provide enough material at the time of graft. In a beagle dog, cryopreservation of BMSCs did not alter the periodontal regeneration compared to the use of non-stored cells (Li et al., 2009). However, additional studies are required to ensure the safety of allogenic grafts and cell cryopreservation.

ADVERSE EFFECTS

With population doublings during the expansion stage, MSCs may be subject to senescence and genetic instability. Progressive shortening of telomeres, modified telomeric structures, and activation of the retinoblastoma protein (pRB) or p53 pathways have been demonstrated to be important triggers for replicative senescence (Schellenberg et al., 2011). Basically, the role of MSCs in cancer can be divided into indirect involvement via the tumor modulatory effect and direct involvement via malignant transformation of themselves (Wong, 2011). The "homing" effect allows MSCs to migrate toward tumor cells, interact with the stroma, and enhance its growth (Wong, 2011). Also, it can lead to malignant transformation of MSCs at the tumor site. While the immunosuppressive properties of MSCs are a good tool for immune disorders, a suppressed immune system may encourage tumor growth in patients with cancer (Shinagawa et al., 2010). To date, no malignant formation has been reported during the course of clinical studies involving periodontal cell therapies (Giordano et al., 2007; Yoshida et al., 2012). Moreover, PDLSC did not induce tumorigenesis after injection into immunodeficient mice for at least 12 weeks (Washio et al., 2010). However, improvements in the understanding of genetic and epigenetic changes will enable researchers to address the risk of tumorigenesis in the context of each type of cell transplantation therapy.

CELL CULTURE DURING LABORATORY PHASES

The definition of MSCs as advanced-therapy medicinal products in European regulations and the US Food and Drug Administration requirements implies the use of production processes in accordance with Good Manufacturing Practices (GMPs). Requirements concern the environment, staff training and qualification, and controls (Sensebe et al., 2013). Culture conditions are not sufficiently developed to mimic the *in-vivo* cell microenvironment and to ensure that cell proliferation and differentiation can be performed safely (van der Sanden et al., 2010).

Cell culture status, such as culture medium composition and oxygen supply, may interact with MSC expansion and *ex-vivo* properties. Even though they are extensively described in studies, there is no consensus about the required culture conditions. Nevertheless, fetal calf serum (FCS), often used as a supplement, contains a xenogeneic source of growth factors and may transmit animal pathogens (Bourin et al., 2010).

Table 2 | Results from animal studies.

References	Experimental group outcomes	Control group outcomes	Conclusion
Akizuki et al., *J. Periodontal. Res.*, 2005	Periodontal tissue healing with bone, cementum, and periodontal ligament formation was observed in three defects. Signs of ankylosis were observed in some specimens.	No cementum was formed, only one defect showed new bone. Parallel connective tissue existed adjacent to the denuded root surface.	The periodontal ligament cell sheet applied in a dehiscence-type defect resulted in regeneration of periodontal tissues in beagle dogs.
Chen et al., *Gene Ther.*, 2008	BMP-2 gene-infected BMSCs Newly formed periodontal ligament fibers were functionally orientated and new connective tissue fibers had been inserted into both the new cementum and the new bone.	BMSCs Woven bone was formed from apical part of defects to middle of the roots. There were small resorption areas with new cementum and fibers.	Regeneration of the periodontal attachment apparatus was enhanced by cells engineered to express BMP-2 gene.
Ding et al., *Stem Cells*, 2010	Both the autologous and allogeneic PDLSC treatments significantly improved periodontal tissue regeneration compared with the HA/TCP and control groups. New bone, cementum, and periodontal ligament were regenerated to normal levels in both the autologous and allogeneic PDLSC groups.	Limited or partial periodontal tissue regeneration in the control groups and HA/TCP group. Little alveolar bone recovery.	A sheet of minipig PDLSCs can repair allogeneic bone defects in an experimental model of periodontitis.
Fawzy El-Sayed et al., *J. Clin. Periodontol.*, 2012	Higher clinical attachment level, probing depth and lower gingival recession. Thin multi-layered squamous sulcular epithelium. Regeneration of bone, cementum, and periodontal ligament with Sharpey's fibers similar to normal periodontium. Higher histological attachment level, lower junctional epithelium length, and connective tissue adhesion.	Thicker multi-layered squamous sulcular epithelium. Periodontal tissue loss, unorganized Sharpey's fibers, root resorption, and ankylosis.	Gingival margin-derived stem/progenitor cells show significant periodontal regenerative potential.
Hasegawa et al., *J. Periodontol.*, 2006	New cementum. New regenerated periodontal ligament separating the new bone from the cementum. No complete alveolar bone reconstruction.	Insufficient periodontal regeneration. Epithelial cells invading top of the furcation, and no cementum regeneration.	Transplanted BMSCs can survive and differentiate into periodontal tissue–composing cells, resulting in enhancement of periodontal tissue regeneration.
Iwata et al., *Biomaterials*, 2009	Complete bone filling with an appropriate space of periodontal ligament was observed. Complete periodontal regeneration with both newly formed bone and cementum connecting with well-oriented collagen fibers.	Almost 50% of bone filling was observed.	Transplantable multi-layered PDLSC cell sheets were successfully fabricated and induced a true periodontal system, including alveolar bone, cementum, and well-oriented fibers at the same time.
Li et al., *Cells Tissues Organs*, 2009	Both cryo- and non-cryopreserved BMSC groups exhibited periodontal regeneration. New PDL was formed between the new alveolar bone and cementum with Sharpey's fibers extending into the newly formed cementum and bone. Cementum and PDL were fully regenerated.	Very little regenerated alveolar bone and cementum. PDL fibers were parallel to the root surface. Small lacunae of resorption were present on roots.	Cryopreserved BMSCs showed no altered regenerative capacity compared with freshly isolated BMSCs in the application of periodontal regeneration.

(Continued)

Table 2 | Continued

References	Experimental group outcomes	Control group outcomes	Conclusion
Liu et al., *Stem Cells*, 2008	New bone and periodontal tissues were regenerated with newly formed Sharpey's fibers anchored into the newly regenerated cementum. Nevertheless, bone was not regenerated to normal level.	Fibers lacking the typical structure of Sharpey's fibers filled in the periodontal defect. Residual inflammation was still present.	The study demonstrated the utility of using an autologous PDLSC therapeutic approach to treat periodontitis in a miniature pig preclinical model.
Nunez et al., *J. Periodontal. Res.*, 2012	Histological characteristics of periodontal regeneration: formation of new cellular cementum, no signs of root resorption or ankylosis, rich capillary vessels. Greater new-bone formation in the PDLDC group.	Healing by repair, with limited formation of new cellular cementum. No signs of root resorption or ankylosis.	Cellular therapy, in combination with a collagen sponge, promoted periodontal regeneration in experimental infra-bony periodontal defects.
Park et al., *Cell Transplant.*, 2011	Healing response was favorable for all treatment groups. For PDLSC group, incremental lines of neocementum were observed, with Sharpey's fibers inserted and cellular cementum at the root apex.	No tissue attachment but presence of surrounding granulation.	PDLSCs may significantly promote periodontal regeneration in class II furcation defects in dogs. Authors suggested PDLSCs were the best candidates for regeneration.
Simsek et al., *Clin. Oral Investig.*, 2012	Formation of new cementum and coronal growth of alveolar bone were observed in all groups. No root resorption or ankylosis was present. No efficacy difference between the groups was found for alveolar bone formation. There was no severe inflammation or swelling and dehiscence of the flaps. Regeneration of cementum for cell group was significantly higher than control group.		Periodontal regeneration with complete filling of class II furcation defects with cementum, alveolar bone, and periodontal ligament was obtained for all groups compared to control group.
Suaid et al., *J. Clin. Periodontol.*, 2011	Woven bone was predominant. In all groups, new cementum and obliquely oriented periodontal fibers were regenerated. Ankylosis was present in one specimen for each group. Nevertheless, cell group presented more new cementum surface, less connective tissue and epithelium along root surface, more bone area than control group.	Large bone marrow spaces were predominant. Down-growth of epithelium was observed in some histological sections.	PDLSCs with guided tissue regeneration were shown to be efficient for periodontal regeneration in class II furcation defects.
Suaid et al., *J. Clin. Periodontol.*, 2012	Cell-treated group exhibited larger area of new bone, more cementum, and more periodontal regeneration than other groups. Complete filling of the furcation was achieved in 2 out of 6 defects.	All defects showed gingival recession with exposure of the furcation area. Defects were incompletely filled, with inflamed connective tissue covered by gingival epithelium. There was no cementum covering entire root area.	PDLSCs in association with guided tissue regeneration may significantly promote periodontal regeneration in class III furcation defects surgically created in dogs.
Takedachi et al., *J. Oral Biosci.*, 2013	Compared to control, bone mineral density increased in 2-wall defects. New bone and new cementum were formed, with connective tissue fibers inserted vertically in the furcation class II defect.		A mix of ASCs and fibrin gel promoted periodontal regeneration in beagle dogs.

(Continued)

Table 2 | Continued

References	Experimental group outcomes	Control group outcomes	Conclusion
Tobita et al., *Cytotherapy*, 2013	New bone was formed and periodontal complex was regenerated after 2 months. Osteocalcin-positive cells were found on the surface of the dentin.	Ingrowth of epithelium into the defect was found in the non-implanted group. Granular tissue and radiolucency were observed in both control groups.	Efficacy of the combination of ASCs and PRP in canine periodontal tissue regeneration.
Tsumanuma et al., *Biomaterials*, 2011	PDLSC group showed more cellular and acellular cementum than other groups. Dense collagen fibers were perpendicularly attached to the cementum-like tissue layer. In BMSC group, fibers were obliquely oriented whereas they were parallel in APC group.	Collagen fibers were sparse. Alveolar bone regeneration was observed in all groups.	PDLSC sheets combined with β-TCP and collagen induced more periodontal regeneration than in other groups.

Abbreviations: ASC, adipose-derived stem cell; BMP2, bone morphogenetic protein-2; BMSC, bone marrow stromal cell; HA, hydroxyapatite; PDL, periodontal ligament; PDLSC, periodontal ligament stromal cell; TCP, tricalcium phosphate.

Addition of human blood-derived products to replace FCS is secured by serologic and nuclear acid testing for blood-transmitted viruses. Human platelet lysate, a blood plasma enriched in platelet growth factors released by freezing-thawing cycles, may be used to produce clinical-grade, stem-cell-loaded biomaterials as an appropriate FCS substitute in line with clinically applicable practice (Warnke et al., 2013). Various other serum-free media based on mixtures of defined growth factors are also able to maintain the main phenotypic and functional characteristics of cultured MSCs (Chase et al., 2010) but are restricted to research purposes and require upgrading for clinical uses.

USE OF CARRIER AND GROWTH FACTORS

In the review of the literature on periodontal regeneration by MSCs, one of the most striking elements is the numerous carriers that have been used. Cortical bone particulates (Simsek et al., 2012), bovine bone (Fawzy El-Sayed et al., 2012), HA (Ding et al., 2010), polymers (Park et al., 2012), collagen (Suaid et al., 2011), hydrogel (Wei et al., 2010), gelatin (Yang et al., 2010), fibrinogen/thrombin (Hynes et al., 2013), PRP (Tobita et al., 2013), cells as sheets (Tsumanuma et al., 2011), alone or in combination, have been suggested to help cell grafting. The heterogeneity among study methodologies makes it difficult to compare them and to draw sound conclusions. Biomolecules, in particular growth factors, have been suggested for addition to scaffolds to enhance periodontal regeneration (Saito et al., 2009). It has been reported that modified MSCs overexpressing growth factors such as BMP-2 (Chen et al., 2008; Chung et al., 2011) or bFGF (Tan et al., 2009) could improve the healing of periodontal defects by maintaining a long-term release of these factors *in situ* (Ramseier et al., 2012).

PERIODONTITIS MODELS IN ANIMALS

Non-human primates are similar to humans, with comparable periodontal tissue structures. However, most non-human primates used for research purposes are expensive and difficult to handle, and ethical restrictions apply to their exploitation (Struillou et al., 2010). The beagle is one of the most commonly used due to its size and its cooperative temperament (Haney et al., 1995; Struillou et al., 2010). Minipigs are also an alternative (Fawzy El-Sayed et al., 2012). However, animal models are unable to mimic some fundamental features: the spontaneous emergence of disease (even though some periodontal lesions can occur in aged animals, periodontal defects are mostly mechanically generated), genetic background, and risk factors (aggressive bacterial flora, occlusal overload, tobacco, prosthesis, systemic diseases of host, and donor, etc.). Overall, these features should be investigated when clinical trials are being designed and analyzed.

REGULATORY INFORMATION

The translation of a cell-based therapy from bench to bedside is challenging, in a regulatory framework involving multiple responsible authorities. Japan, Europe, and the United States have developed quality standards to regulate cell based therapies with Good Clinical Practice and GMP (Yoshida et al., 2012). In the USA, MSCs are considered in the context of "361 Human Cells, Tissues, or Cellular and Tissue-Based Products" (source: www.fda.gov). For European countries, the framework for human trials is fixed by regulation number 1394/2007 on advanced therapy medicinal products, in force since December 2008.

The clinical development plan should start with the submission of a clinical trial authorization application to the competent authority; clinical trials should be designed to demonstrate the safety and efficacy of cells (George, 2011). It can be noted that, in 2006, the WHO stated that all clinical trials should be registered. Moreover, US federal law (Food and Drug Administration) recommends the registration of trials via clinicaltrials.gov to record key elements of study and basic results, and to report adverse events (Califf et al., 2012).

In first-in-man studies, specific safety endpoints may need to be defined to explore: cross-contamination in cases of allogeneic and xenogeneic graft, chromosomal stability, contamination with

microorganisms, safety of engineering devices, stemness potential, functional characterization, and cell phenotype (Dittmar et al., 2010). Because of the risk-benefit balance in periodontal regeneration, emphasis should be placed on the safety of these therapeutics.

CONCLUSION

Animal studies suggest that mesenchymal stem cells are effective and safe for periodontal regeneration. Nevertheless, additional studies are needed to improve periodontal cell therapy regeneration and to decipher the biological mechanisms that are involved. For example, a recent study in dogs suggested that periodontal regeneration could be obtained with stem-cell conditioned medium, possibly thanks to multiple cytokines (Inukai et al., 2013). To materialize the translation of cell therapy from the laboratory to the dental chair a compromise, providing real benefits for patients, respecting biosafety requirements, available at affordable prices, covered by a social security system and sufficiently attractive to encourage industrial companies to invest in its development, is still required. It is possible that cell therapy will be implemented in clinical practice as a routine technique in the future.

ACKNOWLEDGMENTS

This study was supported by internal funding from the University of Toulouse, the Toulouse Faculty of Dentistry, Paul Sabatier University, and Toulouse University Hospital. All authors participated in writing manuscript. The authors thank Susan Becker for proofreading the manuscript.

REFERENCES

Akizuki, T., Oda, S., Komaki, M., Tsuchioka, H., Kawakatsu, N., Kikuchi, A., et al. (2005). Application of periodontal ligament cell sheet for periodontal regeneration: a pilot study in beagle dogs. *J. Periodontal. Res.* 40, 245–251. doi: 10.1111/j.1600-0765.2005.00799.x

Baraniak, P. R., and McDevitt, T. C. (2010). Stem cell paracrine actions and tissue regeneration. *Regen. Med.* 5, 121–143. doi: 10.2217/rme.09.74

Bosshardt, D. D., and Sculean, A. (2009). Does periodontal tissue regeneration really work? *Periodontol. 2000* 51, 208–219. doi: 10.1111/j.1600-0757.2009.00317.x

Bourgeois, D., Llodra, J., Nordblad, A., and Pitts, N. (2005). "Health surveillance in Europe—a selection of essential oral health indicators," in *Technical Report, European Global Oral Health Development Project (EGOHID)*, Lyon.

Bourin, P., Sensebé, L., Planat-Bénard, V., Roncalli, J., Bura-Rivière, A., and Casteilla, L. (2010). Culture and use of mesenchymal stromal cells in phase I and II clinical trials. *Stem Cells Int.* 2010, 503593. doi: 10.4061/2010/503593

Califf, R. M., Zarin, D. A., Kramer, J. M., Sherman, R. E., Aberle, L. H., and Tasneem, A. (2012). Characteristics of clinical trials registered in ClinicalTrials.gov, 2007-2010. *JAMA* 307, 1838–1847. doi: 10.1001/jama.2012.3424

Chase, L. G., Lakshmipathy, U., Solchaga, L. A., Rao, M. S., and Vemuri, M. C. (2010). A novel serum-free medium for the expansion of human mesenchymal stem cells. *Stem Cell Res. Ther.* 1, 8. doi: 10.1186/scrt8

Chen, F. M., and Jin, Y. (2010). Periodontal tissue engineering and regeneration: current approaches and expanding opportunities. *Tissue Eng. Part B Rev.* 16, 219–255. doi: 10.1089/ten.teb.2009.0562

Chen, F. M., Sun, H. H., Lu, H., and Yu, Q. (2012a). Stem cell-delivery therapeutics for periodontal tissue regeneration. *Biomaterials* 33, 6320–6344. doi: 10.1016/j.biomaterials.2012.05.048

Chen, J. S., Wong, V. W., and Gurtner, G. C. (2012b). Therapeutic potential of bone marrow-derived mesenchymal stem cells for cutaneous wound healing. *Front. Immunol.* 3:192. doi: 10.3389/fimmu.2012.00192

Chen, F. M., Wu, L. A., Zhang, M., Zhang, R., and Sun, H. H. (2011). Homing of endogenous stem/progenitor cells for *in situ* tissue regeneration: promises, strategies, and translational perspectives. *Biomaterials* 32, 3189–3209. doi: 10.1016/j.biomaterials.2010.12.032

Chen, Y. L., Chen, P. K., Jeng, L. B., Huang, C. S., Yang, L. C., Chung, H. Y., et al. (2008). Periodontal regeneration using *ex vivo* autologous stem cells engineered to express the BMP-2 gene: an alternative to alveolaplasty. *Gene Ther.* 15, 1469–1477. doi: 10.1038/gt.2008.131

Chung, V. H., Chen, A. Y., Kwan, C. C., Chen, P. K., and Chang, S. C. (2011). Mandibular alveolar bony defect repair using bone morphogenetic protein 2-expressing autologous mesenchymal stem cells. *J. Craniofac. Surg.* 22, 450–454. doi: 10.1097/SCS.0b013e3182077de9

Ding, G., Liu, Y., Wang, W., Wei, F., Liu, D., Fan, Z., et al. (2010). Allogeneic periodontal ligament stem cell therapy for periodontitis in swine. *Stem Cells* 28, 1829–1838. doi: 10.1002/stem.512

Dittmar, K. E., Simann, M., Zghoul, N., Schön, O., Meyring, W., Hannig, H., et al. (2010). Quality of cell products: authenticity, identity, genomic stability and status of differentiation. *Transfus. Med. Hemother.* 37, 57–64. doi: 10.1159/000284401

Dominici, M., Le Blanc, K., Mueller, I., Slaper-Cortenbach, I., Marini, F., Krause, D., et al. (2006). Minimal criteria for defining multipotent mesenchymal stromal cells. the international society for cellular therapy position statement. *Cytotherapy* 8, 315–317. doi: 10.1080/14653240600855905

Esposito, M., Grusovin, M. G., Papanikolaou, N., Coulthard, P., and Worthington, H. V. (2009). Enamel matrix derivative (Emdogain(R)) for periodontal tissue regeneration in intrabony defects. *Cochrane Database Syst. Rev.* 7:CD003875. doi: 10.1002/14651858.CD003875.pub3

Fawzy El-Sayed, K. M., Paris, S., Becker, S. T., Neuschl, M., De Buhr, W., Salzer, S., et al. (2012). Periodontal regeneration employing gingival margin-derived stem/progenitor cells: an animal study. *J. Clin. Periodontol.* 39, 861–870. doi: 10.1111/j.1600-051X.2012.01904.x

Feng, F., Akiyama, K., Liu, Y., Yamaza, T., Wang, T. M., Chen, J. H., et al. (2010). Utility of PDL progenitors for *in vivo* tissue regeneration: a report of 3 cases. *Oral Dis.* 16, 20–28. doi: 10.1111/j.1601-0825.2009.01593.x

Feng, F., and Hou, L. T. (1992). Treatment of osseous defects with fibroblast-coated hydroxylapatite particles. *J. Formos. Med. Assoc.* 91, 1068–1074.

Feng, F., Liu, C. M., Hsu, W. C., and Hou, L. T. (1995). Long-term effects of gingival fibroblast-coated hydroxylapatite graft on periodontal osseous defects. *J. Formos. Med. Assoc.* 94, 494–498.

George, B. (2011). Regulations and guidelines governing stem cell based products: clinical considerations. *Perspect. Clin. Res.* 2, 94–99. doi: 10.4103/2229-3485.83228

Giordano, A., Galderisi, U., and Marino, I. R. (2007). From the laboratory bench to the patient's bedside: an update on clinical trials with mesenchymal stem cells. *J. Cell. Physiol.* 211, 27–35. doi: 10.1002/jcp.20959

Haney, J. M., Zimmerman, G. J., and Wikesjo, U. M. (1995). Periodontal repair in dogs: evaluation of the natural disease model. *J. Clin. Periodontol.* 22, 208–213. doi: 10.1111/j.1600-051X.1995.tb00136.x

Hare, J. M., Fishman, J. E., Gerstenblith, G., Difede Velazquez, D. L., Zambrano, J. P., Suncion, V. Y., et al. (2012). Comparison of allogeneic vs autologous bone marrow-derived mesenchymal stem cells delivered by transendocardial injection in patients with ischemic cardiomyopathy: the POSEIDON randomized trial. *JAMA* 308, 2369–2379. doi: 10.1001/jama.2012.25321

Hasegawa, N., Kawaguchi, H., Hirachi, A., Takeda, K., Mizuno, N., Nishimura, M., et al. (2006). Behavior of transplanted bone marrow-derived mesenchymal stem cells in periodontal defects. *J. Periodontol.* 77, 1003–1007. doi: 10.1902/jop.2006.050341

Hoogduijn, M. J., and Dor, F. J. (2013). Mesenchymal stem cells: are we ready for clinical application in transplantation and tissue regeneration? *Front. Immunol.* 4:144. doi: 10.3389/fimmu.2013.00144

Hou, L. T., Tsai, A. Y., Liu, C. M., and Feng, F. (2003). Autologous transplantation of gingival fibroblast-like cells and a hydroxylapatite complex graft in the treatment of periodontal osseous defects: cell cultivation and long-term report of cases. *Cell Transplant.* 12, 787–797. doi: 10.3727/000000003108747262

Huang, G. T., Gronthos, S., and Shi, S. (2009). Mesenchymal stem cells derived from dental tissues vs. those from other sources: their biology and role in regenerative medicine. *J. Dent. Res.* 88, 792–806. doi: 10.1177/0022034509340867

Hynes, K., Menicanin, D., Han, J., Marino, V., Mrozik, K., Gronthos, S., et al. (2013). Mesenchymal stem cells from iPS cells facilitate periodontal regeneration. *J. Dent. Res.* 92, 833–839. doi: 10.1177/0022034513498258

Ilic, D., and Polak, J. (2012). Stem cell based therapy–where are we going? *Lancet* 379, 877–878. doi: 10.1016/S0140-6736(12)60155-X

Inukai, T., Katagiri, W., Yoshimi, R., Osugi, M., Kawai, T., Hibi, H., et al. (2013). Novel application of stem cell-derived factors for periodontal regeneration. *Biochem. Biophys. Res. Commun.* 430, 763–768. doi: 10.1016/j.bbrc.2012.11.074

Iwata, T., Yamato, M., Tsuchioka, H., Takagi, R., Mukobata, S., Washio, K., et al. (2009). Periodontal regeneration with multi-layered periodontal ligament-derived cell sheets in a canine model. *Biomaterials* 30, 2716–2723. doi: 10.1016/j.biomaterials.2009.01.032

Kern, S., Eichler, H., Stoeve, J., Kluter, H., and Bieback, K. (2006). Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. *Stem Cells* 24, 1294–1301. doi: 10.1634/stemcells.2005-0342

Li, H., Yan, F., Lei, L., Li, Y., and Xiao, Y. (2009). Application of autologous cryopreserved bone marrow mesenchymal stem cells for periodontal regeneration in dogs. *Cells Tissues Organs* 190, 94–101. doi: 10.1159/000166547

Li, J., Ezzelarab, M. B., and Cooper, D. K. (2012). Do mesenchymal stem cells function across species barriers? Relevance for xenotransplantation. *Xenotransplantation* 19, 273–285. doi: 10.1111/xen.12000

Lin, N. H., Gronthos, S., and Mark Bartold, P. (2009). Stem cells and future periodontal regeneration. *Periodontol. 2000* 51, 239–251. doi: 10.1111/j.1600-0757.2009.00303.x

Liu, Y., Zheng, Y., Ding, G., Fang, D., Zhang, C., Bartold, P. M., et al. (2008). Periodontal ligament stem cell-mediated treatment for periodontitis in miniature swine. *Stem Cells* 26, 1065–1073. doi: 10.1634/stemcells.2007-0734

Mizuno, H., Kagami, H., Mase, J., Mizuno, D., and Ueda, M. (2010). Efficacy of membranous cultured periosteum for the treatment of patients with severe periodontitis: a proof-of-concept study. *Nagoya J. Med. Sci.* 72, 59–70.

Needleman, I. G., Worthington, H. V., Giedrys-Leeper, E., and Tucker, R. J. (2006). Guided tissue regeneration for periodontal infra-bony defects. *Cochrane Database Syst. Rev*. 19:CD001724. doi: 10.1002/14651858.CD001724.pub2

Nunez, J., Sanz-Blasco, S., Vignoletti, F., Munoz, F., Arzate, H., Villalobos, C., et al. (2012). Periodontal regeneration following implantation of cementum and periodontal ligament-derived cells. *J. Periodontal. Res.* 47, 33–44. doi: 10.1111/j.1600-0765.2011.01402.x

Okuda, K., Yamamiya, K., Kawase, T., Mizuno, H., Ueda, M., and Yoshie, H. (2009). Treatment of human infrabony periodontal defects by grafting human cultured periosteum sheets combined with platelet-rich plasma and porous hydroxyapatite granules: case series. *J. Int. Acad. Periodontol.* 11, 206–213.

Oliver, R. C., Brown, L. J., and Loe, H. (1998). Periodontal diseases in the United States population. *J. Periodontol.* 69, 269–278. doi: 10.1902/jop.1998.69.2.269

Park, C. H., Rios, H. F., Jin, Q., Sugai, J. V., Padial-Molina, M., Taut, A. D., et al. (2012). Tissue engineering bone-ligament complexes using fiber-guiding scaffolds. *Biomaterials* 33, 137–145. doi: 10.1016/j.biomaterials.2011.09.057

Park, J. Y., Jeon, S. H., and Choung, P. H. (2011). Efficacy of periodontal stem cell transplantation in the treatment of advanced periodontitis. *Cell Transplant.* 20, 271–285. doi: 10.3727/096368910X519292

Pihlstrom, B. L., Michalowicz, B. S., and Johnson, N. W. (2005). Periodontal diseases. *Lancet* 366, 1809–1820. doi: 10.1016/S0140-6736(05)67728-8

Poncelet, A. J., Denis, D., and Gianello, P. (2009). Cellular xenotransplantation. *Curr. Opin. Organ Transplant.* 14, 168–174. doi: 10.1097/MOT.0b013e3283292522

Ramseier, C. A., Rasperini, G., Batia, S., and Giannobile, W. V. (2012). Advanced reconstructive technologies for periodontal tissue repair. *Periodontol. 2000* 59, 185–202. doi: 10.1111/j.1600-0757.2011.00432.x

Saito, A., Saito, E., Handa, R., Honma, Y., and Kawanami, M. (2009). Influence of residual bone on recombinant human bone morphogenetic protein-2-induced periodontal regeneration in experimental periodontitis in dogs. *J. Periodontol.* 80, 961–968. doi: 10.1902/jop.2009.080568

Schellenberg, A., Lin, Q., Schuler, H., Koch, C. M., Joussen, S., Denecke, B., et al. (2011). Replicative senescence of mesenchymal stem cells causes DNA-methylation changes which correlate with repressive histone marks. *Aging (Albany NY)* 3, 873–888.

Sensebe, L., Gadelorge, M., and Fleury-Cappellesso, S. (2013). Production of mesenchymal stromal/stem cells according to good manufacturing practices: a review. *Stem Cell Res. Ther.* 4, 66. doi: 10.1186/scrt217

Sensebe, L., Krampera, M., Schrezenmeier, H., Bourin, P., and Giordano, R. (2010). Mesenchymal stem cells for clinical application. *Vox Sang.* 98, 93–107. doi: 10.1111/j.1423-0410.2009.01227.x

Shin, L., and Peterson, D. A. (2013). Human mesenchymal stem cell grafts enhance normal and impaired wound healing by recruiting existing endogenous tissue stem/progenitor cells. *Stem Cells Transl. Med.* 2, 33–42. doi: 10.5966/sctm.2012-0041

Shinagawa, K., Kitadai, Y., Tanaka, M., Sumida, T., Kodama, M., Higashi, Y., et al. (2010). Mesenchymal stem cells enhance growth and metastasis of colon cancer. *Int. J. Cancer* 127, 2323–2333. doi: 10.1002/ijc.25440

Simsek, S. B., Keles, G. C., Baris, S., and Cetinkaya, B. O. (2012). Comparison of mesenchymal stem cells and autogenous cortical bone graft in the treatment of class II furcation defects in dogs. *Clin. Oral Investig.* 16, 251–258. doi: 10.1007/s00784-010-0486-7

Sorrell, J. M., and Caplan, A. I. (2010). Topical delivery of mesenchymal stem cells and their function in wounds. *Stem Cell Res. Ther.* 1, 30. doi: 10.1186/scrt30

Struillou, X., Boutigny, H., Soueidan, A., and Layrolle, P. (2010). Experimental animal models in periodontology: a review. *Open Dent. J.* 4, 37–47. doi: 10.2174/1874210601004010037

Suaid, F. F., Ribeiro, F. V., Gomes, T. R., Silverio, K. G., Carvalho, M. D., Nociti, F. H. Jr., et al. (2012). Autologous periodontal ligament cells in the treatment of Class III furcation defects: a study in dogs. *J. Clin. Periodontol.* 39, 377–384. doi: 10.1111/j.1600-051X.2012.01858.x

Suaid, F. F., Ribeiro, F. V., Rodrigues, T. L., Silverio, K. G., Carvalho, M. D., Nociti, F. H., et al. (2011). Autologous periodontal ligament cells in the treatment of class II furcation defects: a study in dogs. *J. Clin. Periodontol.* 38, 491–498. doi: 10.1111/j.1600-051X.2011.01715.x

Takedachi, M., Sawada, K., Yamamoto, S., Ozasa, M., Shimabukuro, Y., Kitamura, M., et al. (2013). Periodontal tissue regeneration by transplantation of adipose tissue-derived stem cells. *J. Oral Biosci.* 55, 137–142. doi: 10.1016/j.job.2013.04.004

Tan, Z., Zhao, Q., Gong, P., Wu, Y., Wei, N., Yuan, Q., et al. (2009). Research on promoting periodontal regeneration with human basic fibroblast growth factor-modified bone marrow mesenchymal stromal cell gene therapy. *Cytotherapy* 11, 317–325. doi: 10.1080/14653240902824757

Tobita, M., Uysal, C. A., Guo, X., Hyakusoku, H., and Mizuno, H. (2013). Periodontal tissue regeneration by combined implantation of adipose tissue-derived stem cells and platelet-rich plasma in a canine model. *Cytotherapy* doi: 10.1016/j.jcyt.2013.05.007. [Epub ahead of print].

Tsumanuma, Y., Iwata, T., Washio, K., Yoshida, T., Yamada, A., Takagi, R., et al. (2011). Comparison of different tissue-derived stem cell sheets for periodontal regeneration in a canine 1-wall defect model. *Biomaterials* 32, 5819–5825. doi: 10.1016/j.biomaterials.2011.04.071

van der Sanden, B., Dhobb, M., Berger, F., and Wion, D. (2010). Optimizing stem cell culture. *J. Cell. Biochem.* 111, 801–807. doi: 10.1002/jcb.22847

Warnke, P. H., Humpe, A., Strunk, D., Stephens, S., Warnke, F., Wiltfang, J., et al. (2013). A clinically-feasible protocol for using human platelet lysate and mesenchymal stem cells in regenerative therapies. *J. Craniomaxillofac. Surg.* 41, 153–161. doi: 10.1016/j.jcms.2012.07.003

Washio, K., Iwata, T., Mizutani, M., Ando, T., Yamato, M., Okano, T., et al. (2010). Assessment of cell sheets derived from human periodontal ligament cells: a pre-clinical study. *Cell Tissue Res.* 341, 397–404. doi: 10.1007/s00441-010-1009-1

Wei, N., Gong, P., Liao, D., Yang, X., Li, X., Liu, Y., et al. (2010). Auto-transplanted mesenchymal stromal cell fate in periodontal tissue of beagle dogs. *Cytotherapy* 12, 514–521. doi: 10.3109/14653241003709702

Wong, R. S. (2011). Mesenchymal stem cells: angels or demons? *J. Biomed. Biotechnol.* 2011:459510. doi: 10.1155/2011/459510

Wu, Y., Chen, L., Scott, P. G., and Tredget, E. E. (2007). Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. *Stem Cells* 25, 2648–2659. doi: 10.1634/stemcells.2007-0226

Yamada, Y., Ueda, M., Hibi, H., and Baba, S. (2006). A novel approach to periodontal tissue regeneration with mesenchymal stem cells and platelet-rich plasma using tissue engineering technology: a clinical case report. *Int. J. Periodontics Restorative Dent.* 26, 363–369.

Yang, Y., Rossi, F. M., and Putnins, E. E. (2010). Periodontal regeneration using engineered bone marrow mesenchymal stromal cells. *Biomaterials* 31, 8574–8582. doi: 10.1016/j.biomaterials.2010.06.026

Yoshida, T., Washio, K., Iwata, T., Okano, T., and Ishikawa, I. (2012). Current status and future development of cell transplantation therapy for periodontal tissue regeneration. *Int. J. Dent.* 2012, 307024. doi: 10.1155/2012/307024

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 13 September 2013; paper pending published: 05 October 2013; accepted: 19 October 2013; published online: 15 November 2013.

*Citation: Trofin EA, Monsarrat P and Kémoun P (2013) Cell therapy of periodontium: from animal to human? Front. Physiol. **4**:325. doi: 10.3389/fphys.2013.00325*

This article was submitted to Craniofacial Biology, a section of the journal Frontiers in Physiology.

II.2.2 Principaux éléments de discussion et perspectives

A propos du modèle animal

Les lésions parodontales ont été créées de manière mécanique (fraises chirurgicales) dans 74% des articles ou entretenues par un processus inflammatoire dans 21% des cas par apport d'un fil de ligature, de gutta-percha ou de matériau d'empreinte. Les modèles utilisés étaient donc peu représentatifs de la physiopathologie de la maladie parodontale humaine. Pourtant, 49% des études utilisaient des chiens et 14% des cochons nains. La morphologie des dents et l'anatomie parodontale sont proches de celles de l'Homme, et des parodontites apparaissent spontanément lors de l'avancée en âge. Comment alors expliquer que malgré ces avantages, les lésions soient créées à la fraise sur des animaux jeunes ? Le tarif important pour obtenir et élever les animaux, la difficulté d'obtenir des lésions naturelles reproductibles et standardisées, la nécessité d'obtenir des résultats et des publications rapidement, pourraient l'expliquer. L'utilisation de modèles murins de parodontite par gavage oral bactérien est donc nécessaire pour déchiffrer les mécanismes de la régénération, tester différentes modalités thérapeutiques, avant de transposer cela vers des modèles animaux plus couteux (85).

A propos des biomatériaux utilisés

De nombreux biomatériaux ont été utilisés comme vecteur cellulaire pour des résultats variables. Les résultats sont exprimés en différence moyenne standardisée, c'est-à-dire que l'absence d'effet CSMs versus contrôle pourrait être due soit à une absence d'effet des CSMs soit à un effet trop fort du biomatériau porteur que l'on retrouve dans les deux groupes. Ainsi, les CSMs ont démontré dans notre analyse un bénéfice significativement supérieur au contrôle, pour les substituts osseux, pour le collagène, gélatine, acide hyaluronique comme matériaux porteurs. Au contraire, l'utilisation de polymères, de dérivés plaquettaires et de dérivés de la matrice amélaire ne démontre pas de bénéfice de la thérapie cellulaire. L'hypothèse envisagée est que l'utilisation du matériau porteur doit être fonction de l'anatomie de la lésion. Son rôle doit être de garder les cellules sur site. En effet, une lésion rétentive (lésion à 2 ou 3 parois) supportera l'utilisation de structures gel ou liquide, alors que des lésions à 1 paroi ou inter radiculaires nécessiteraient plus de maintien sur le site.

A propos des sources de cellules

Il n'y avait pas de différence significative en termes de régénération de cément ou d'os alvéolaire en fonction du type de CSMs. Des études effectuant des comparaisons *in vivo* entre les différents types de CSMs (surtout d'origine intra-buccale versus extra-orale) sont donc nécessaires pour confirmer ces résultats. Cela signifierait que le tissu adipeux, par son accessibilité et sa disponibilité, la faible co –morbidité de son prélèvement, aurait un avantage majeur.

A propos de la méthodologie

La moitié des études seulement était randomisée et seule une étude avait réalisé un calcul du nombre d'animaux nécessaire (86). Lors de l'étape initiale, 6 études ont été exclues car les données du groupe contrôle n'étaient pas fournies. Une analyse méthodologique plus approfondie par l'intermédiaire de la grille de lecture animale ARRIVE a révélé que de nombreux éléments étaient manquants dans les publications finales. Les méthodologies d'analyse statistique manquaient de détails, la présence ou l'absence d'évènements indésirables n'était pas reportée dans 45% des études ; ceci d'autant plus important que deux résumés de conférence rapportent des formations tumorales après implantation de CSMs du ligament parodontal dans des lésions parodontales. Même si ces évènements semblent rares et isolés, il est fondamental que tout évènement indésirable soit signalé. Les titres n'avaient pas assez de détails, notamment concernant le type de cellules employées et le modèle animal choisi.

Les auteurs ont mesuré des données cliniques dans 37% des études (ex. profondeur de poche, niveau d'attache), des données radiologiques dans 14% des études (ex. densité osseuse) et des données histomorphométriques dans 91% des études (ex. épaisseur du cément, niveau épithélial, formation osseuse). Très peu de données biologiques chiffrées étaient disponibles (ex. identification et quantification des populations cellulaires). Ainsi, d'autres mesures pourraient être réalisées pour optimiser les coupes histologiques comme la localisation des cellules greffées dans le prélèvement, l'organisation du ligament parodontal, la caractérisation des progéniteurs *in situ* ou la distribution de la néovascularisation.

Depuis la réalisation de cette revue systématique, d'autres études ont été publiées dans le champ de la régénération parodontale par CSMs. Parmi ces études, deux retiennent particulièrement

l'attention. Tout d'abord, des CSMs dérivées d'iPS ont été utilisées chez le rat pour régénérer des lésions induites par des ligatures au niveau de la première molaire maxillaire avec une inoculation dans la cavité orale de *Porphyromonas gingivalis* (87). Dans un modèle de lésions de type fenestration mécanique chez le rat (88), la greffe d'ASCs a également démontré un bénéfice significativement supérieur au contrôle en terme de formation d'os alvéolaire et d'épaisseur de cément.

Tous ces éléments convergent vers la nécessité d'étudier plus en détail l'effet des ASCs sur la régénération parodontale en utilisant des modèles animaux plus proches de la physiopathologie de la parodontite humaine. Il est important que des quantifications soient systématiquement réalisées au niveau des études histologiques, en particulier au niveau de la vascularisation et de l'organisation de l'appareil desmodontal.

II.2.3 Expérimentation chez la souris

II.2.3.1 Article 6: "Periodontal tissue regeneration using syngeneic adipose-derived mesenchymal stromal cells in a mouse model"

Le travail présenté dans cette partie fait l'objet d'un article en cours de soumission dans « Stem Cells Translational Medicine ».

Contexte

Nous avons démontré précédemment la complexité des mécanismes physiopathologiques impliqués et intriqués dans l'évolution des parodontites, que ce soit des déterminants locaux, systémiques ou psychologiques. Etudier la régénération parodontale nécessite donc d'avoir des modèles animaux dont l'écologie immunitaire et microbienne tente de mimer celle retrouvée chez l'Homme. Or nous avons clairement montré la très faible représentativité des modèles animaux actuellement utilisés pour la régénération parodontale par CSMs, et le manque d'évaluation quantitative de la qualité de l'architecture du parodonte profond néoformé.

L'objectif principal de cette étude est de déterminer si l'utilisation syngénique d'ASCs dans un modèle murin de parodontite permet la régénération d'un appareil parodontal fonctionnel.

Méthodologie

Pour ces raisons, un modèle original de lésion parodontale chez la souris (85), induit par gavage oral de bactéries parodonto-pathogènes a été utilisé. Un mélange de bactéries parodonto-pathogènes (*Porphyromonas gingivalis*, *Fusobacterium nucleatum* et *Prevotella intermedia*) a été apporté de manière répétée pendant 1 mois au niveau des régions molaires pour induire les lésions parodontales (85). Afin de pouvoir tracer le devenir local des cellules greffées, les ASCs ont été obtenues de souris transgéniques C57Bl6/J GFP positives et transplantées à des souris sauvages C57Bl6/J chez lesquelles les lésions parodontales avaient été générées. Les ASCs ont été apportés dans une solution de collagène d'un côté, ou seulement la solution de collagène en controlatéral. 6 souris ont été sacrifiées aux temps 0 puis à 1, 6 et 12 semaines. Cinq souris non colonisées par la solution de parodonto-pathogènes, ont également été utilisées en contrôle.

Au niveau des données histomorphométriques, ont été évalués : la régénération osseuse (distance amélo-cémentaire – crête alvéolaire), la régénération cémentaire, le nombre de vaisseaux et l'organisation parodontale. Ainsi, en plus du modèle en soi, l'originalité de cette étude provient de la quantification de la vascularisation et de l'analyse mathématique que nous avons réalisées sur les fibres parodontales. Pour ce paramètre une méthode mathématique de transformée de Hough (HT) a été mise en œuvre grâce au logiciel Matlab 2012 (Mathworks, France). La figure ci-dessous (Figure II-5) résume les différentes étapes nécessaires. A partir des coupes histologiques colorées au trichrome de Masson, la composante rouge uniquement a été extraite, donnant la meilleure netteté des fibres desmodontales. En utilisant un cadre de taille calibrée, les zones correspondant aux fibres obliques et aux fibres horizontales du ligament ont été découpées. Les lignes ont été détectées (filtre « canny »), et l'image soumise à l'HT. La matrice de HT représente les probabilités de présence des lignes dans un plan polaire (angle, distance). La distribution de probabilité de leur orientation est donnée par histogramme. L'entropie de cette distribution est calculée (mesure statistique de l'aspect aléatoire de la distribution). Une diminution de l'entropie implique des fibres mieux orientées (89).

Résultats

Une semaine après la greffe des ASC-GFP+, les cellules se retrouvent localisées au sein du ligament parodontal. 12 semaines après la greffe, les cellules ne sont plus visibles au sein du tissu mais une augmentation du dépôt cémentaire et du nombre de vaisseaux, une amélioration de l'organisation du desmodonte sont retrouvés au niveau des sites greffés par rapport au contrôle. Nous avons également montré la présence de populations cellulaires spécifiques

recrutées au niveau des sites expérimentaux, pouvant évoquer le recrutement de progéniteurs in-situ par les ASCs notamment de progéniteurs ostéo-cémentogéniques.

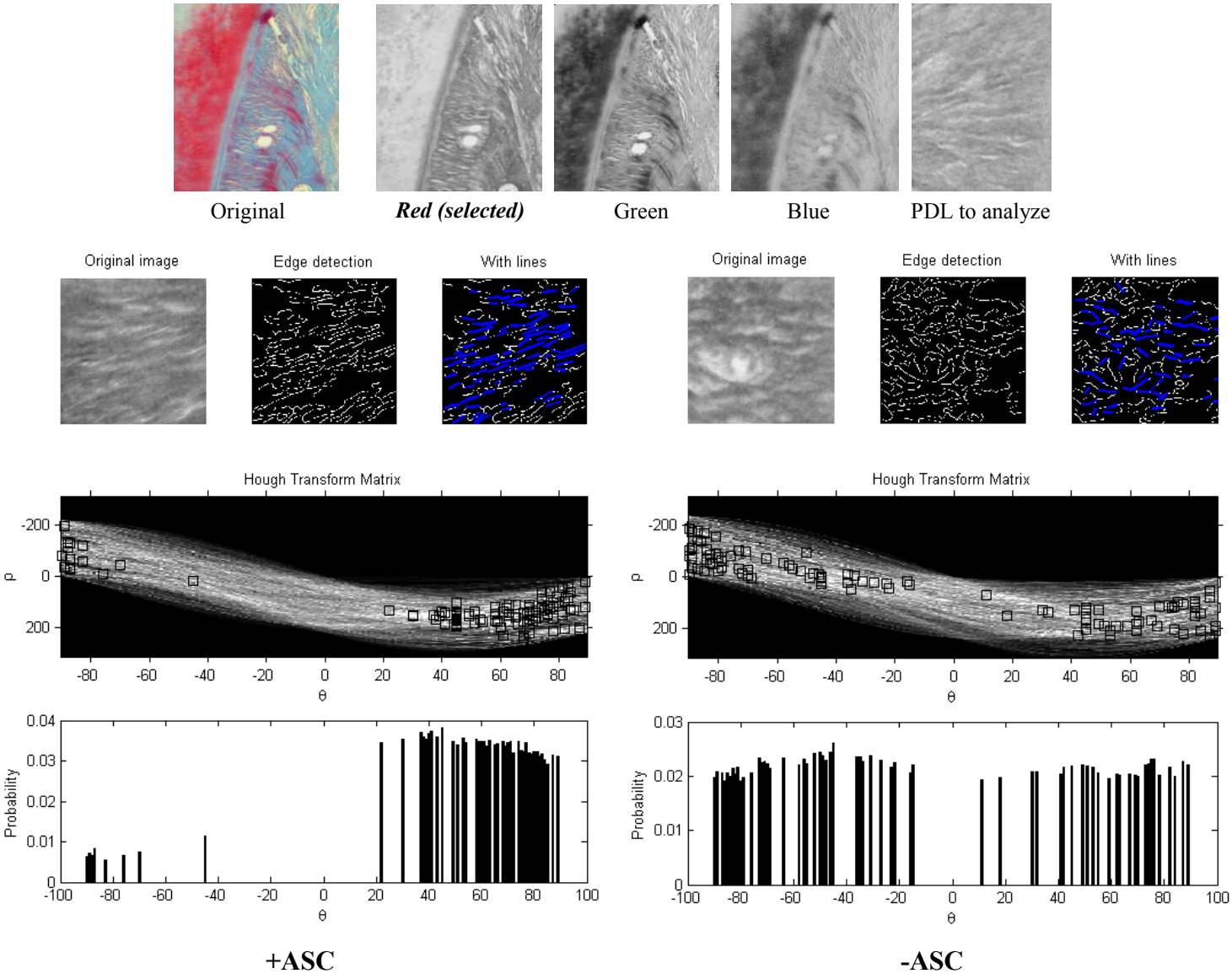


Figure II-5 : Les différentes étapes nécesaires pour la caractérisation de l'organisation des fibres desmodontales.

Article

Periodontal tissue regeneration using syngeneic adipose-derived stromal cells in a mouse model

Journal:	*Stem Cells Translational Medicine*
Manuscript ID	SCTM-16-0028
Manuscript Type:	Original Research
Date Submitted by the Author:	15-Jan-2016
Complete List of Authors:	Lemaitre, Mathieu; Faculté de chirurgie dentaire, Département de biologie; UMR 5273, U1031, STROMALab; University of Toulouse; CNRS; EFS; INSERM Monsarrat, Paul; Faculté de chirurgie dentaire; UMR 5273, U1031, STROMALab; University of Toulouse; CNRS; EFS; INSERM Blasco, Vincent; Faculté de chirurgie dentaire; UMR 1048, I2MC; University of Toulouse UPS; INSERM Loubières, Pascale; Faculté de chirurgie dentaire; UMR 1048, I2MC; University of Toulouse UPS; INSERM Burcelin, Rémy; UMR 1048, I2MC; University of Toulouse UPS; INSERM Casteilla, Louis; CNRS 5273; UMR STROMALab; Université de Toulouse; UPS; INSERM U1031; EFS Pyrénées – Méditerranée; BP 84225, F-31 432 Planat-Benard, Valerie; UMR5273 UPS, CNRS, Inserm U1031, STROMALab Kémoun, Philippe; Toulouse University Hospital - CHU de Toulouse, ; Toulouse Faculty of Dentistry - Paul Sabatier University, Department of Biology
Keywords:	Mesenchymal Stromal Cells, Mesenchymal Stem Cell Transplantation, Mice, Periodontitis, Regenerative Medicine, Subcutaneous Fat

Periodontal tissue regeneration using syngeneic adipose-derived stromal cells in a mouse model

Running title

Periodontal regeneration by adipose stem cells

Authors

Mathieu Lemaitre [a,b,*], Paul Monsarrat [b,c,*], Vincent Blasco-Baque [a,d], Pascale Loubières [a,d], Rémy Burcelin [d], Louis Casteilla [b], Valérie Planat-Bénard [b] and Philippe Kémoun [a]

Affiliations

a. University of Toulouse, Dental Faculty, Department of Biological Sciences, Toulouse University Hospital, Toulouse, France.
b. UMR 5273, U1031, STROMALab; University of Toulouse; CNRS; EFS; INSERM; Toulouse, France.
c. University of Toulouse, Dental Faculty, Department of Anatomical Sciences and Radiology, Toulouse University Hospital, Toulouse, France.
d. UMR 1048, I2MC; University of Toulouse UPS; INSERM; Toulouse, France.

*: These authors contributed equally to this work

1- Mathieu Lemaitre, DDS : E-mail address : mathieu.lemaitre@inserm.fr
2- Paul Monsarrat, DDS: E-mail address: paul.monsarrat@gmail.com
3- Vincent Blasco-Baque, PhD, DDS: E-mail address: vincent.blasco@inserm.fr
4- Pascale Loubières, Lab Technician. E-mail address: pascale.loubieres@inserm.fr
5- Rémy Burcelin, PhD: E-mail address: remy.burcelin@inserm.fr
6- Louis Casteilla, PhD: E-mail address: louis.casteilla@inserm.fr
7- Valérie Planat-Bénard, PhD: E-mail address: valerie.planat@inserm.fr
8- Philippe Kémoun, PhD, DDS (*Corresponding author*):
Mail address : UFR Odontologie de Toulouse
3, chemin des maraîchers
31062 Toulouse Cedex 9.
Telephone number: +33 5 62 17 29 29
Fax number: +33 5 61 25 47 19
E-mail address: philippe.kemoun@univ-tlse3.fr

Word count: 3573

Abstract

Introduction. Current treatment of periodontitis is still associated with a high degree of variability in clinical outcomes. Recent advances in regenerative medicine by mesenchymal cells, including adipose stromal cells (ASC) have paved the way to improved periodontal regeneration (PD) but little is known about the biological processes involved. Here, we aimed to use syngeneic ASCs for periodontal regeneration in a new, relevant, bacteria-induced periodontitis model in mice. **Methods.** Periodontal defects were induced in female C57BL6/J mice by oral gavage with periodontal pathogens. $2x10^5$ syngeneic mouse ASCs expressing GFP (GFP+/ASC) were grafted within a collagen vehicle in the lingual part of the first lower molar periodontium (experimental) while carrier alone was implanted in the contralateral side (control). Animals were sacrificed 0, 1, 6 and 12 weeks after treatment by GFP+/ASC or vehicle graft, and microscopic examination, immunofluorescence, and innovative bio-informatics histomorphometry methods were used to reveal deep periodontium changes. **Results.** From one to six weeks after surgery, GFP+ cells were identified in the periodontal ligament (PDL), in experimental sites only. After 12 weeks, cementum regeneration, the organization of PDL fibers, the number of PD vessels, and bone morphogenetic protein (BMP)-2 and osteopontin (OPN) expression were greater in experimental sites than in controls. Specific stromal cell subsets were recruited in the newly formed tissue in ASC-implanted periodontium only. **Discussion.** These data suggest that ASC grafting in diseased deep periodontium relevant for human pathology induces a significant improvement of the PDL microenvironment, leading to a recovery of tooth-supporting tissue homeostasis.

MeSH terms

Mesenchymal Stromal Cells, Mesenchymal Stem Cell Transplantation, Mice, Periodontitis, Regenerative Medicine, and Subcutaneous Fat

Abbreviations

α-MEM: alpha Minimum Essential Medium
ASC: Adipose-derived Mesenchymal Stromal Cell
BM-MSC: Bone-Marrow-Mesenchymal Stromal Cell
BMP: Bone Morphogenetic Protein
CEJ: Cemento-Enamel Junction
CFU: Colony Forming Unit
EC: Endothelial Cell
GFP: Green Fluorescent Protein
HT: Hough Transform
IF: Immunofluorescence
MSC: Mesenchymal Stromal Cell
OPN: Osteopontin
PBS: Phosphate-Buffer Saline
PDL: Periodontal Ligament
RT: Room Temperature

Introduction

Periodontitis is a chronic immuno-infectious disease, characterized by loss of the tissues supporting the teeth, and leading to or aggravating systemic disorders such as diabetes, polyarthritis or atherosclerosis [1]. The defects result from a local homeostasis disruption caused by both the virulence of a periodontal pathogenic microflora [2] and an inappropriate immune response [3, 4]. From a pathophysiology point of view [3, 4], the destruction of deep periodontium tissues (i.e. root cementum, periodontal ligament (PDL) and alveolar bone) induces the formation of crevices called "periodontal pockets" between the tooth root and its bony socket [5], leading to tooth loss.

Periodontal regeneration aims to restore both the architecture and function of tooth supporting tissues through the recruitment and activation of endogenous progenitors, especially those expressing CD146 markers [5], leading to renewal of the connective attachment underlying the new junctional epithelium. The restitution of dense connective fibers of PDL, anchored between the newly formed alveolar bone and root cementum, is critical for the long-term prognosis [6, 7]. A broad range of periodontal regenerative procedures has been proposed, including guided tissue regeneration, enamel matrix derived proteins (EMD), platelet-rich plasma, and bone graft, but these procedures have been reported to lack efficiency and mainly result in incomplete defect reconstruction and poor reproducibility [8, 9]. Persistence of low-grade inflammation and infection, poor dental plaque control, blood clot stability, and systemic diseases may be involved in these unpredictable outcomes by preventing the activities of periodontal progenitors *in situ* [10].

Regenerative treatment of connective tissues therefore aims to create a micro-environment suitable for the migration, proliferation and commitment of endogenous mesenchymal progenitors towards specific differentiation cell-phenotypes involved in the synthesis of

extracellular matrix components, such as bone morphogenetic proteins (BMP) or osteopontin (OPN) [6]. Recent advances in regenerative medicine and the biology of mesenchymal stem cells have paved the way to new strategies based on tissue engineering [11]. By their capacity to differentiate and acquire different phenotypes, to be stimulated by the local micro-environment, and to exhibit paracrine potential (e.g. mitogenic, angiogenic, anti-apoptotic, immunomodulatory factor), exogenous MSC would favor the production of new tissues, by their own action or by stimulating the activity of endogenous progenitors [12, 13]. MSC can potentially be isolated from almost all organs [14] and are commonly purified from bone marrow, adipose tissue and umbilical cord. For many reasons related to safety in tissue sample processing, access to cell sources and availability, adipose-derived mesenchymal stromal cells (ASCs) are expected to be a valuable source of cells and are being increasingly tested at clinical level [15, 16].

A previous systematic review of the literature demonstrated the efficacy and safety of oral or extra-oral MSCs (including ASCs) to regenerate periodontal tissues [17], but most of the studies were performed on poorly relevant defect models [17]. Animal periodontal defects were usually induced mechanically using dental burs, with or without additional procedures (ligature or impression paste to favor bacterial colonization) [17-19] that did not create lesions or a tissue environment close to the pathophysiology of periodontitis.

In this study, we aimed to avoid such limitations by using a model with periodontal lesions induced by oral gavage with periopathogens, which led to periodontal defects relevant to human pathophysiology [17, 18]. In this context, we investigated the use of syngeneic ASCs that expressed the green fluorescent protein (GFP) for *in situ* tracking and pointed out their ability to enhance deep periodontal tissue wound healing using classic and innovative bioinformatics measurements.

Material and methods

Periodontitis mouse model

The periodontitis model in mice was induced as already described [20]. This protocol was in accordance with the ARRIVE guidelines for reporting animal research [21]. All procedures performed on mice were approved by the local ethics committees of Toulouse University Hospital and INSERM under the authorization number "C3155507". C57BL6/J wild-type female mice (Charles River, L'Arbresle, France) were group-housed (five per cage) in a specific pathogen-free controlled environment with inverted 12-hr daylight cycle in our animal facilities. Drinking water was supplemented with sulfamethoxazole (200 mg/5 mL) and trimethoprime (40 mg/5 mL) 10 days before bacterial oral gavage at a daily dose of 95 mg/kg.

Under isoflurane anesthesia at 8 weeks of age, the mice received 1 ml of a mix of 10^9 CFU of *Porphyromonas gingivalis* (ATCC 33277), *Fusobacterium nucleatum* and *Prevotella intermedia* previously identified [18] in 2% carboxymethylcellulose in the molar regions. This step was repeated 4 times a week for 1 month to induce periodontal lesions.

Isolation of GFP+ ASCs

Transgenic C57BL6/J mice constitutively expressing green fluorescent protein (GFP) were anesthetized by intraperitoneal administration of 100 mg/kg ketamine (Merial, Gerland, France) and 10 mg/kg xylazin (Bayer, Puteaux, France). Inguinal subcutaneous adipose tissues were processed as previously described to isolate ASCs [22]. Briefly, inguinal adipose tissues were digested at 37°C for 45 minutes in phosphate-buffered saline (PBS) containing 2% bovine serum albumin and 2 mg/mL collagenase 1 (Sigma-Aldrich, France), filtrated at 25 µm, then centrifuged at 600g for 10 minutes, to remove mature adipocytes. Red blood cells

were lysed into buffer containing 140 mM NH_4Cl and 20 mM Tris for 5 minutes at 4°C. Cells were centrifuged at 600g for 5 minutes and the vascular stromal fraction was seeded at $30x10^3$ cells/cm^2 in DMEM-F12 medium supplemented with 10% newborn calf serum, 0.25 µg/mL amphotericin, 100 µg/mL streptomycin and 100 UI/mL penicillin, and maintained in a 5% CO_2 atmosphere.

Cell grafting into mouse periodontium

At 80% confluence, GFP+/ASC (Passage 1) were trypsinized, counted, washed once in PBS, then used for transplantation. A gingival lingual flap was performed under binocular microscopy in the first lower molar region. A split mouth design was employed: on one side, $2x10^5$ ASCs were applied using 2% type I collagen as the carrier, and the other side was used as a control, treated with the vehicle only. A total of 24 mice (48 periodontal defects) were used, distributed over 4 time points (0, 1, 6 and 12 weeks).

Optical microscopic examination and measurements

At the end of each time interval, mice were anesthetized and sacrificed by cervical dislocation; mandibles were collected, fixed in 4% formaldehyde, embedded in paraffin then cut at 4 µm using a microtome (Jung 2055 Autocut, Leica, France). Sections were stained with Masson's trichrome and photographed under a light microscope equipped with a Nikon CoolPix 4500. Bone regeneration was assessed by measuring the distance between the cemento-enamel junction (CEJ) and the top of the alveolar crest. A frame of 1000 pixels² (px^2) surface area, representative of the cementum defect after 4 weeks of periopathogen infection was drawn downward from the CEJ to the remaining cementum. An example of cementum measurement in an animal at 0 weeks (day of surgery) is provided in Fig. S1. The number of vessels inside the PDL was also counted. Each measurement was performed twice

(blinded to previous assessment), on at least 5 sections per periodontal defect, and the mean of these 5 measurements was considered.

Immunofluorescence analyses

Immunofluorescence analyses were used to investigate the distribution of mineralized tissue markers (BMP-2 and OPN) and connective progenitor subsets. Because endogenous GFP expression was too weak (data not shown) to highlight implanted cells, a rabbit alexa-488 anti-GFP was used to localize grafted GFP+/ASC (Table S1). Paraffin was removed with xylene, and sections were rehydrated using a descending ethanol series. For the detection of intracellular markers only, permeabilization was performed first, using Triton X100 in PBS at 0.1% for 15 min. Antigens were unmasked by incubation in citrate buffer (10 mM, pH 6.0) in an 80°C water bath for 20 min. Saturation of non-specific sites was achieved by incubating the sections for 15 min at RT in PBS containing 5% normal serum from the same species as the host of the secondary antibody. Primary antibodies were used at the specified concentration (Table S1) for 2 hours in a humidified chamber at room temperature (RT) for surface markers, or overnight at 4°C for intracellular targets. Slides were rinsed three times for 5 min in PBS containing 0.2% Tween 20. Secondary antibodies were then used at the specified concentration (Table S1) for 1 hour at RT, then washed. Slides were mounted using ProLong© containing Hoechst (Invitrogen, France) and photographed by epifluorescence microscopy (Nikon Eclipse T2000).

Hough transform analyses to quantify entropy of PDL fibers

A measure of the PDL fiber architecture was achieved using the Hough transform (HT). Images were first oriented vertically using a line tangent to the root. The red component of the color image was kept to better visualize the fibers. We submitted the oblique and horizontal

fibers of PDL to HT using Matlab 2012 software (Mathworks, France). Fig. S2 summarizes the processing steps. Using HT, lines corresponding to the main directions of detected fibers were drawn. The probability for each angular direction was derived from the Hough transform matrix and plotted as a histogram distribution. The entropy of this distribution was computed, which provided a statistical measure of randomness. A decrease of this parameter means that fibers are better orientated [23].

Statistical analyses

An analysis of variance with some random effect was used to determine whether a difference between the experimental side and the control side could be detected in non-colonized tissues, at 0 weeks (baseline), 6 weeks or 12 weeks. Parameters in the experimental side were compared to the corresponding side of non-colonized and 0 week mice, corrected by multiple comparisons using the Bonferroni adjustment. The level of significance was set to 0.05. Graphics and statistics were performed using Stata 13.1 (StataCorp, Tx, USA).

Results

Grafted GFP-expressing ASC were identified in PDL from 1 to 6 weeks after surgery

During the course of the periodontal wound healing, we used IF microscopy to follow the distribution and fate of grafted GFP+/ASC (Fig. 1a-f). From one to 6 weeks after surgery, ASC were localized only in the experimental site close to the wound bed near the CEJ, toward the apical part of the PDL, and surrounding PDL and alveolar bone blood vessels (Fig. 1 a-c). Cells expressing the GFP marker were almost undetectable at ASC implanted sites after 12-weeks (Fig. 1d) and at control-treated sites (Fig. 1e).

ASC grafting enhanced cementum regeneration, PDL fiber organization and number of vessels.

Fig. 2 is representative of the differences between experimental and control sites regarding their cementum appearance, PDL fiber organization and re-vascularization, 12 weeks after treatment of diseased deep periodontium by ASC or vehicle. Overall, compared to controls, ASC-grafted sites exhibited higher cementum deposition, enhanced periodontal fiber organization, with denser Sharpey's fibers, and an increase in PDL vascularization.

From 6 weeks after treatment, cementum and PDL regeneration occurred in both control and experimental sites (Figs. 3 and 4). Microscopic examination of 12-weeks ASC-treated periodontium showed that newly deposited cementum-like tissue was thicker than at contralateral vehicle-only grafted sites, and was similar to healthy cementum (Fig. 3a-d), as confirmed by histomorphometry. This demonstrated that the cementum thickness was entirely recovered only on experimental sides (Fig. 3e; $p<0.001$). The amount of cementum regeneration increased over time and was significantly higher that at the starting point (0 week).

Fig. 4 illustrates the stronger PDL fiber organization (orientation, length and density) in the experimental compared to the control side. Sharpey anchorage appeared denser and more homogenous in ASC grafted PDL tissues than in controls. As for cementum regeneration, PDL microscopic appearance was close to that of healthy structures in experimental sites but not in vehicle-only treated sites (Fig. 4 a-d). The Hough transform (HT) was used to quantify oblique and horizontal fiber organization by determination of the structure entropy (Fig. S2). Twelve weeks after treatment, the entropy of both oblique and horizontal PDL fibers was significantly lower in experimental than in control sites, suggesting that the ASC graft enhanced periodontal connective attachment regeneration (Fig. 4 e-f). Interestingly, fiber

entropy time-decay was confirmed, and fiber organization completely rescued in ASC implanted sites only.

The microscopic evaluation of PDL vascularization showed that grafting ASC promoted a significant increase in the number of both small- and large-diameter PDL vessels, from two to four-fold compared to control at 6 and 12 weeks (Fig. 5 a-b), in PDL-alveolar bone at 6 weeks (Fig. 5c) and in gingiva at 6 and 12 weeks (Fig. 5d). Six weeks after treatment, the number of PDL vessels was greater than at the starting point and in healthy tissues but decreased later.

Taken together, these data indicate that cementum regeneration, PDL fiber organization and PDL vessel number were improved in experimental relative to control sites.

Deep periodontium BMP2 and OPN expression is modified by ASC graft

To investigate the distribution of non-collagen matrix markers during the periodontal regeneration, the change in BMP-2 and OPN expression was analyzed by immunofluorescence microscopy (Fig. 6). One week after treatment, BMP-2 staining was mainly identified in the cervical part of the PDL in both vehicle- and ASC-grafted tissues (Fig. 6 a, d). Six weeks after surgery, BMP-2 expression was stronger in ASC-grafted than in control sites, and extended toward the apical part of the PDL on the experimental side only (Fig. 6 g, j). Twelve weeks after challenge, the expression of BMP-2 had returned to normal in experimental and control sites (Fig. 6 m, p).

During the course of periodontal tissue regeneration with or without ASC grafting, OPN expression underscored that cementum deposition and was clearly more enhanced in experimental sites than in controls. As for BMP-2 expression, OPN staining highlighted the PDL reorganization (Fig 6. b, e, h, k, n, q).

PDL examination revealed OPN and BMP-2 co-localization (Fig 6. c, f, i, l, o, r), but strong OPN deposition only sustained the PDL/cementum and PDL/alveolar bone interfaces compared to BMP-2 staining.

ASC graft impacted the PDL expression of SCA-1 and CD146 during periodontal healing

Next, we used IF to compare the expression of SCA-1 and CD146, two surface markers for connective tissue progenitors, in healing periodontal tissues with or without ASC grafting (Fig. 7). SCA-1 and CD146 expression in the PDL cell population were clearly modified by ASC implantation from one to 12 weeks after surgery, compared to vehicle-only treated sites.

One week after treatment of mouse altered periodontium by syngeneic ASC, numerous distinct SCA-1+/CD146- and SCA-1 -/ CD146+ cell populations were localized in the alveolar bone side of the PDL, mainly surrounding blood vessels, while these cell subsets were hardly seen in control PDL (Fig. 7 a, b). After 6 weeks, experimental sites exhibited SCA-1-/ CD146+ PDL cells underlining the putative cementoblast layer, while SCA-1+/CD146- subsets remained located around PDL vessels. Interestingly, a transient peri-vascular SCA-1+/CD146+ PDL cell population emerged at this stage, and was completely lacking at control sites (Fig. 7 c, d). Finally, in 12-weeks challenged animals, SCA-1 expression had almost entirely disappeared in PDL. The SCA-1-/CD146+ PDL cell population remained highlighted in the PDL/alveolar bone interface in ASC grafted sites only (Fig. 7 e, f).

Discussion

Two key elements are required to consider periodontal cell-based therapy: the most relevant pre-clinical model of human disease, and *ad integrum* restitution of *de novo* cementum,

alveolar bone and functional PDL [7]. We addressed both issues and our data point out that syngeneic exogenous ASC may be very useful in periodontal regeneration [18]. In a murine model relevant for human tooth-supporting tissue pathophysiology and after ASC transplantation, we highlighted structural and functional changes occurring in deep periodontal tissues during the regeneration process. Moreover, our data show, for the first time, that ASC graft enhances all the deep periodontium healing: cementum regeneration as well as PDL organization, neocapillarization, and expression of progenitor/matrix markers.

A complete cementum recovery is crucial to sustain a long-term favourable outcome because it is essential for strong anchoring of Sharpey PDL fibers in the root and, thus, the maintaining of the tooth in its socket [24]. Cementum tissue regeneration results from complex matrix-to-cells and cell-to-cell interactions. Such cementum regeneration is clearly enhanced by ASC grafting, in line with a recent study in dogs [25]. The improvement we report here of OPN and BMP-2 expression in the experimental side confirms the positive effect of ASC grafting in cementum regeneration. This is consistent with the important role of OPN in the recruitment and maintenance of selective cells at the root surface [24]. This non-collagenous protein is involved in the organization of mineral and organic phases (collagen meshwork), bound to collagen matrix via $\alpha_V\beta_3$ integrin [24, 26]. Moreover, OPN expression is critical for local innate immunity, inducing macrophage recruitment on site and tissue remodelling [26]. Such an increase in OPN has been documented in a periodontal fenestration rat model where periodontal cells were grafted [27].

Surprisingly, we did not find any marked effect of ASC in alveolar bone regeneration (Fig. S3). Alveolar bone regeneration by cell therapy is controversial and depends on the animal species, the defect designs, and the sources and carriers of grafted cells. For example, enhancement of new alveolar bone formation has been obtained in rats after ASC periodontal cell therapy (mechanically created bone fenestration) [28] but no significant alveolar bone

regeneration was reported after the grafting of iPSC-derived MSCs in rat periodontal lesions (induced by ligature and *Porphyromonas gingivalis* infection) [29]. Conversely to infra-bony defects, well-known to be regenerated with high predictability, our periodontitis-pathogen-induced alveolar bone defect model had a horizontal shape. Thus, we can hypothesize that the morphology of these defects could be unsuitable to assess the effect of ASC grafting in alveolar bone reconstruction.

The periodontal ligament contains several cell populations including fibroblasts, MSC, sensory system, vascularization and collagenous and non-collagenous matrix proteins [30, 31]. Fibre orientation and density encourage a nurturing role for cementum, a uniform distribution of masticatory forces and a remodelling of the alveolar bone [30]. The present results suggest that ASC use may enhance the formation of new functional PDL, by the increase of well-oriented oblique and horizontal fibers [17]. Moreover, BMP-2 distribution was found to emphasise the reorganization and orientation of PDL fibers during the periodontal wound healing, and was transiently up-regulated in ASC-treated sides compared to controls. In a rat fenestration model, regeneration of PDL fibers, well-orientated perpendicularly to the root surface, was observed after bone marrow-MSC grafting [32]. Moreover, in an acute rat rotator cuff repair model, with special focus on the healing of the tendon-to-bone insertion, grafted ASC in a collagen carrier led to significantly more elastic and less scarred newly formed tissue compared to control [33]. The use of MSC for scarring in aged mice demonstrated increased wound tensile strength [34]. Altogether, these data strongly suggest that ASC therapy enhances collagen fiber re-organization during PDL wound healing.

Because the blood supply is critical for optimal wound healing, we investigated the impact of ASC use in periodontal neo angiogenesis. As described in a murine skin wound healing model [35], an increase in the number PDL vessels was shown at the cell-treated site. Angiogenesis

is crucial to supply oxygen and nutrients to support periodontal wound healing. Interestingly, both endogenous and grafted MSC were found to be located around blood vessels, as already reported [35], suggesting cross talk between mesenchymal progenitors and endothelial cells (EC). ASC have been reported to stabilize EC networks by enhancing pericyte properties, thus improving vascular network formation [36, 37]. Moreover, pericytes are suggested to share MSC features and may be involved in tissue regeneration/repair by differentiation toward specialized connective phenotypes [38, 39]. ASCs have already been suggested to be involved in neocapillarization by a direct trans-differentiation in CD31-expressing EC [35], or via a vascular endothelial growth factor *in situ* secretion [35, 40].

Regeneration of connective tissue is mainly based on the activation of specific signaling pathways involved in the recruitment and mobilization of endogenous MSC in the wound bed [41]. However, especially in periodontal wound healing, the regeneration process may be incomplete and/or inappropriate because current therapy does not focus enough on endogenous MSC recruitment/commitment [42, 43]. Thus, to highlight the role of grafted, exogenous ASC on periodontal progenitor recruitment from PDL substratum, we investigated the fate of mesenchymal cells from the deep periodontium compartment using CD146 [5] and SCA-1 as an osteo-cementogenic precursors marker and a well-known mouse MSC/ASC marker, respectively [44, 45]. Although these markers may also be expressed by subsets of grafted murine ASC [38, 39, 46], the progressive fade-out of grafted cells conversely to the increase of CD146 and SCA-1 expression in PDL strongly suggests that implanted cells do not themselves differentiate toward specialized target phenotypes but rather induce a micro-environment suitable for progenitor recruitment from substratum. ASC graft *in situ* activities may be mediated via a paracrine effect, although their progressive phasing in implanted sites could also provide signalling for the local environment as already reported [47, 48].

Six weeks after implantation, experimental sites exhibited SCA-1 positive cells, some of which expressed CD146, surrounding blood vessels. Because SCA-1 was shown to characterize undifferentiated mesenchymal pools [49], this paravascular cell population, mainly emerging in grafted sites, may be considered as early periodontal precursors. Moreover, our results point out that a CD146+ SCA-1 - cell subpopulation is located under the cementoblast layer. This subset may be regarded as a pre-differentiated population, already committed to the cementoblastic lineage [5]. Overall, these data suggest that ASC grafting may enhance the recruitment and commitment of endogenous periodontal progenitors that correlate with the promotion of deep periodontal tissue regeneration, as previously reported in other applications of ASC therapy [50-55].

Altogether, our data show that ASC grafting significantly supports the periodontal regeneration linked to enhanced cementum regeneration, PDL fiber organization and number of vessels. These data suggest that ASC-cell grafting could be a future clinical therapy for periodontal disease.

Acknowledgments

This study was supported by funding from the Midi-Pyrenees region, Paul Sabatier University, Toulouse University Hospital and the research platform of Toulouse Dental Faculty. The authors thank Susan Becker for proofreading the manuscript, Florence Capilla (histopathological research platform, CPTP, Toulouse Purpan), Marion Taurand (STROMALab) and the research platform of Toulouse Dental Faculty for their technical help, and Professor Gerard Brunel for his help in the study design and for his constant inspiration.

Disclosure of Interest

The authors declare no conflict of interest.

References

1. Albandar J. Periodontal disease surveillance. **J Periodontol**. 2007;78:1179-1181.

2. Socransky SS, Haffajee AD, Cugini MA et al. Microbial complexes in subgingival plaque. **J Clin Periodontol**. 1998;25:134-144.

3. Darveau RP. Periodontitis: a polymicrobial disruption of host homeostasis. **Nat Rev Microbiol**. 2010;8:481-490.

4. Armitage GC. Learned and unlearned concepts in periodontal diagnostics: a 50-year perspective. **Periodontol 2000**. 2013;62:20-36.

5. Kemoun P, Gronthos S, Snead ML et al. The role of cell surface markers and enamel matrix derivatives on human periodontal ligament mesenchymal progenitor responses in vitro. **Biomaterials**. 2011;32:7375-7388.

6. Hynes K, Menicanin D, Gronthos S et al. Clinical utility of stem cells for periodontal regeneration. **Periodontol 2000**. 2012;59:203-227.

7. Bosshardt DD, Sculean A. Does periodontal tissue regeneration really work? **Periodontol 2000**. 2009;51:208-219.

8. Esposito M, Grusovin MG, Papanikolaou N et al. Enamel matrix derivative (Emdogain(R)) for periodontal tissue regeneration in intrabony defects. **Cochrane Database Syst Rev**. 2009:CD003875.

9. Needleman IG, Worthington HV, Giedrys-Leeper E et al. Guided tissue regeneration for periodontal infra-bony defects. **Cochrane Database Syst Rev**. 2006:CD001724.

10. Chen FM, Zhang J, Zhang M et al. A review on endogenous regenerative technology in periodontal regenerative medicine. **Biomaterials**. 2010;31:7892-7927.

11. Vasu S, Leitman SF, Tisdale JF et al. Donor demographic and laboratory predictors of allogeneic peripheral blood stem cell mobilization in an ethnically diverse population. **Blood**. 2008;112:2092-2100.

12. Murphy MB, Moncivais K, Caplan AI. Mesenchymal stem cells: environmentally responsive therapeutics for regenerative medicine. **Exp Mol Med**. 2013;45:e54.

13. Shin L, Peterson DA. Human mesenchymal stem cell grafts enhance normal and impaired wound healing by recruiting existing endogenous tissue stem/progenitor cells. **Stem Cells Transl Med**. 2013;2:33-42.

14. Dominici M, Le Blanc K, Mueller I et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. **Cytotherapy**. 2006;8:315-317.

15. Bourin P, Bunnell BA, Casteilla L et al. Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint statement of the International Federation for Adipose Therapeutics (IFATS) and Science and the International Society for Cellular Therapy (ISCT). **Cytotherapy**. 2013.

16. Nicoletti GF, De Francesco F, D'Andrea F et al. Methods and procedures in adipose stem cells: state of the art and perspective for translation medicine. **J Cell Physiol**. 2015;230:489-495.

17. Monsarrat P, Vergnes JN, Nabet C et al. Concise Review: Mesenchymal Stromal Cells Used for Periodontal Regeneration: A Systematic Review. **Stem Cells Transl Med**. 2014.

18. Blasco-Baque V, Serino M, Vergnes JN et al. High-fat diet induces periodontitis in mice through lipopolysaccharides (LPS) receptor signaling: protective action of estrogens. **PLoS One**. 2012;7:e48220.

19. Graves DT, Fine D, Teng YT et al. The use of rodent models to investigate host-bacteria interactions related to periodontal diseases. **J Clin Periodontol**. 2008;35:89-105.

20. Blasco-Baque V, Kemoun P, Loubieres P et al. [Impact of periodontal disease on arterial pressure in diabetic mice]. **Ann Cardiol Angeiol (Paris)**. 2012;61:173-177.

21. Kilkenny C, Browne WJ, Cuthill IC et al. Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research. **J Pharmacol Pharmacother**. 2010;1:94-99.

22. Planat-Benard V, Silvestre JS, Cousin B et al. Plasticity of human adipose lineage cells toward endothelial cells: physiological and therapeutic perspectives. **Circulation**. 2004;109:656-663.

23. Bayan C, Levitt JM, Miller E et al. Fully automated, quantitative, noninvasive assessment of collagen fiber content and organization in thick collagen gels. **J Appl Phys**. 2009;105:102042.

24. Arzate H, Zeichner-David M, Mercado-Celis G. Cementum proteins: role in cementogenesis, biomineralization, periodontium formation and regeneration. **Periodontol 2000**. 2015;67:211-233.

25. Tobita M, Uysal CA, Guo X et al. Periodontal tissue regeneration by combined implantation of adipose tissue-derived stem cells and platelet-rich plasma in a canine model. **Cytotherapy**. 2013.

26. Giachelli CM, Steitz S. Osteopontin: a versatile regulator of inflammation and biomineralization. **Matrix Biol**. 2000;19:615-622.

27. Lekic PC, Rajshankar D, Chen H et al. Transplantation of labeled periodontal ligament cells promotes regeneration of alveolar bone. **Anat Rec**. 2001;262:193-202.

28. Akita D, Morokuma M, Saito Y et al. Periodontal tissue regeneration by transplantation of rat adipose-derived stromal cells in combination with PLGA-based solid scaffolds. **Biomed Res**. 2014;35:91-103.

29. Yang H, Aprecio RM, Zhou X et al. Therapeutic effect of TSG-6 engineered iPSC-derived MSCs on experimental periodontitis in rats: a pilot study. **PLoS One**. 2014;9:e100285.

30. Beertsen W, McCulloch CA, Sodek J. The periodontal ligament: a unique, multifunctional connective tissue. **Periodontol 2000**. 1997;13:20-40.

31. Lorimier S, Kemoun P. Histophysiology of the periodontium [Histophysiologie du parodonte]. **Médecine buccale**. 2012;[54751].

32. Yang Y, Rossi FM, Putnins EE. Periodontal regeneration using engineered bone marrow mesenchymal stromal cells. **Biomaterials**. 2010;31:8574-8582.

33. Valencia Mora M, Antuna Antuna S, Garcia Arranz M et al. Application of adipose tissue-derived stem cells in a rat rotator cuff repair model. **Injury**. 2014;45 Suppl 4:S22-27.

34. Lee S, Szilagyi E, Chen L et al. Activated mesenchymal stem cells increase wound tensile strength in aged mouse model via macrophages. **J Surg Res**. 2013;181:20-24.

35. Ebrahimian TG, Pouzoulet F, Squiban C et al. Cell therapy based on adipose tissue-derived stromal cells promotes physiological and pathological wound healing. **Arterioscler Thromb Vasc Biol**. 2009; 29:503-510.

36. Rohringer S, Hofbauer P, Schneider KH et al. Mechanisms of vasculogenesis in 3D fibrin matrices mediated by the interaction of adipose-derived stem cells and endothelial cells. **Angiogenesis**. 2014;17:921-933.

37. Andreeva ER, Pugach IM, Gordon D et al. Continuous subendothelial network formed by pericyte-like cells in human vascular bed. **Tissue Cell**. 1998;30:127-135.

38. Crisan M, Yap S, Casteilla L et al. A perivascular origin for mesenchymal stem cells in multiple human organs. **Cell Stem Cell**. 2008;3:301-313.

39. Caplan AI. All MSCs are pericytes? **Cell Stem Cell**. 2008;3:229-230.

40. Olea FD, Locatelli P, Hnatiuk A et al. Vascular endothelial growth factor overexpression does not enhance adipose stromal cell-induced protection on muscle damage in critical limb ischemia. **Arterioscler Thromb Vasc Biol**. 2015;35:184-188.

41. Chen FM, Wu LA, Zhang M et al. Homing of endogenous stem/progenitor cells for in situ tissue regeneration: Promises, strategies, and translational perspectives. **Biomaterials**. 2011;32:3189-3209.

42. Foster BL, Popowics TE, Fong HK et al. Advances in defining regulators of cementum development and periodontal regeneration. **Curr Top Dev Biol**. 2007;78:47-126.

43. Bartold PM, Shi S, Gronthos S. Stem cells and periodontal regeneration. **Periodontol 2000**. 2006;40:164-172.

44. Gronthos S, Zannettino AC, Graves SE et al. Differential cell surface expression of the STRO-1 and alkaline phosphatase antigens on discrete developmental stages in primary cultures of human bone cells. **J Bone Miner Res**. 1999;14:47-56.

45. Holmes C, Stanford WL. Concise review: stem cell antigen-1: expression, function, and enigma. **Stem Cells**. 2007;25:1339-1347.

46. Gil-Ortega M, Garidou L, Barreau C et al. Native adipose stromal cells egress from adipose tissue in vivo: evidence during lymph node activation. **Stem Cells**. 2013;31:1309-1320.

47. Eggenhofer E, Luk F, Dahlke MH et al. The life and fate of mesenchymal stem cells. **Front Immunol**. 2014;5:148.

48. Bai X, Yan Y, Coleman M et al. Tracking long-term survival of intramyocardially delivered human adipose tissue-derived stem cells using bioluminescence imaging. **Mol Imaging Biol**. 2011;13:633-645.

49. San Miguel SM, Fatahi MR, Li H et al. Defining a visual marker of osteoprogenitor cells within the periodontium. **J Periodontal Res**. 2010;45:60-70.

50. Salgado AJ, Reis RL, Sousa NJ et al. Adipose tissue derived stem cells secretome: soluble factors and their roles in regenerative medicine. **Curr Stem Cell Res Ther**. 2010;5:103-110.

51. Im GI. Endogenous Cartilage Repair by Recruitment of Stem Cells. **Tissue Eng Part B Rev**. 2015.

52. Du L, Yang P, Ge S. Stromal cell-derived factor-1 significantly induces proliferation, migration, and collagen type I expression in a human periodontal ligament stem cell subpopulation. **J Periodontol**. 2012;83:379-388.

53. Kimura Y, Komaki M, Iwasaki K et al. Recruitment of bone marrow-derived cells to periodontal tissue defects. **Front Cell Dev Biol**. 2014;2:19.

54. Song M, Heo J, Chun JY et al. The paracrine effects of mesenchymal stem cells stimulate the regeneration capacity of endogenous stem cells in the repair of a bladder-outlet-obstruction-induced overactive bladder. **Stem Cells Dev**. 2014;23:654-663.

55. Di Rocco G, Gentile A, Antonini A et al. Enhanced healing of diabetic wounds by topical administration of adipose tissue-derived stromal cells overexpressing stromal-derived factor-1: biodistribution and engraftment analysis by bioluminescent imaging. **Stem Cells Int**. 2010;2011:304562.

Legends of Figures

Fig. 1: Localization of the grafted GFP+ ASCs. Cells were tracked by immunofluorescence. (a) GFP+/ASC were identified in the experimental, periodontium-implanted site at 1 week, not only close to the wound bed near the cervical part but also toward the apical part of the PDL and surrounding the ligament and alveolar bone blood vessels. (b, c) GFP+/ASC localization surrounding PDL and alveolar bone blood vessels (b) and in the apical part of the PDL (c). (d) Undistinguishable GFP+/ASC in the grafted side at week 12. (e) Undistinguishable cells in vehicle-only treated control sites. AB: alveolar bone, D: dentin, DP: dental pulp, DR: dental root, G: gingiva, PDL: periodontal ligament. Bar: 100 µm

Fig. 2: ASC graft improved deep periodontium regeneration. Histological section of mouse deep periodontium 12 weeks after vehicle (a) or ASC (b) grafting. Cementum deposition (blue arrow), PDL fiber organization (black arrows), and number of vessels (*) had increased in the experimental, ASC-treated side compared to the control side. CEJ: Cemento-enamel junction. AB: alveolar bone, D: dentin, PDL: periodontal ligament. Bar: 50 µm

Fig. 3: ASC graft improved cementum regeneration. Histological sections of healthy (a), and diseased cementum before treatment (0 week (wk), (b)) and 12-week vehicle-only (c) or ASC (d) treated tissue. (e): Histomorphometry analysis of cementum deposition. The area of cementum was measured in square pixels in the control side (white bars) and the ASC grafted side (gray bars). 12 weeks after grafting, the cementum was rescued in the ASC treated side

only. CEJ: Cemento-enamel junction. C: cementum, AB: alveolar bone, D: dentin, PDL: periodontal ligament. Bar: 50 µm. The "b" code indicates a significant difference in the treatment side between each time point and 0 week (baseline). The "n" code indicates a significant difference of the treatment side at each time point and not colonized. "#", "##" and "###" indicate a significant difference between treatment and control sides with $p<0.05$, $p<0.01$ and $p<0.001$ respectively.

Fig. 4: ASC graft improved PDL fiber re-organization. (a - d): Histological sections of healthy (a) and diseased PDL fibers before treatment (0 week (wk), (b)) and 12-weeks vehicle (c) or ASC (d) treated. (e – f): Histomorphometry analysis of PDL. 12 weeks after treatment, the entropy calculated from the Hough transform was significantly lower in ASC grafted sites than controls, for both horizontal (e) and oblique (f) fibers, and close to that of healthy PDL. The "b" code indicates a significant difference in the treatment side between each time point and 0 week (baseline). The "n" code indicates a significant difference in the treatment side at each time point and not colonized. "#", "##" and "###" indicate a significant difference between treatment and control sides with $p<0.05$, $p<0.01$ and $p<0.001$ respectively. C: cementum, AB: alveolar bone, D: dentin, PDL: periodontal ligament. Bar: 50 µm.

Fig. 5: ASC graft enhanced PDL neo-vascularization. (a - d) Six weeks after treatment, the number of >15µm diameter (a), <15µm diameter (b), alveolar bone (c) and gingival (d) PDL vessels was significantly higher in ASC-treated than vehicle-treated sites. The "b" code indicates a significant difference of the treatment side between each time point and 0 week (baseline). The "n" code indicated a significant difference of the treatment side at each time

point and not colonized. "#", "##" and "###" indicate a significant difference between treatment and control sides with $p<0.05$, $p<0.01$ and $p<0.001$ respectively.

Fig. 6: Effect of ASC graft on BMP2 and OPN expression during periodontal healing. BMP2 (green, a, d, g, j, m, p) and OPN staining (magenta, b, e, h, k, n, q), and their co-localization (c, f, I, l, o, r) 1, 6 and 12 weeks after deep periodontium grafting +/- ASC. Six weeks after surgery, BMP-2 expression extended toward the apical part of the PDL in experimental side only. OPN expression underlined the cementum deposition and was clearly enhanced by ASC implantation compared to control. Cell nuclei in blue. AB: alveolar bone, D: dentin, GE: gingival epithelium, PDL: periodontal ligament. Bar: 100 µm

Fig. 7: ASC graft impacted the PDL expression of SCA-1 and CD146 during periodontal healing. Co-localization of SCA-1 (green) and CD146 (magenta) expression 1 (a, b), 6 (c, d) and 12 (e, f) weeks after deep periodontium grafting +/- ASC. After 6 weeks, ASC periodontium implantation only clearly promoted the emergence of SCA-1+/CD146- (green arrows) and SCA-1+/CD146+ (white arrows) populations in peri-vascular locations, and an SCA-1-/CD146+ (magenta arrows) subset indicating the cementum-lining cells. Cell nuclei in blue. AB: alveolar bone, D: dentin, GE: gingival epithelium, PDL: periodontal ligament. DP: dental pulp. Bar: 50 µm.

Fig. S1: Example of cementum measurement. In untreated periodontitis-induced mice (0 week, day 0 surgery), a frame of 1000 pixels' height was drawn downward the CEJ to the remaining cementum surface area, here in dotted lines, to quantify the cementum defect.

AB: alveolar bone, C: cementum, CEJ: cemento-enamel junction, D: dentin, GE: gingival epithelium, PDL: periodontal ligament.

Fig. S2: Measure of the periodontal ligament organization by entropy. Red components of histological sections were extracted, edges were detected and the Hough Transform matrix was employed to select lines corresponding to the main directions of detected fibers. The probability for each angular direction was plotted as a histogram. The entropy of this distribution was computed, which provided a statistical measure of randomness.

Fig. S3: No effect of ASC for alveolar bone regeneration. Results from histomorphometry analysis of alveolar bone. The distance between the alveolar crest and the cemento-enamel junction was measured in pixels in the control side (white bars) and the grafted side (gray bars). The “n” code indicated a significant difference of the treatment side between each time point and baseline and not colonized.

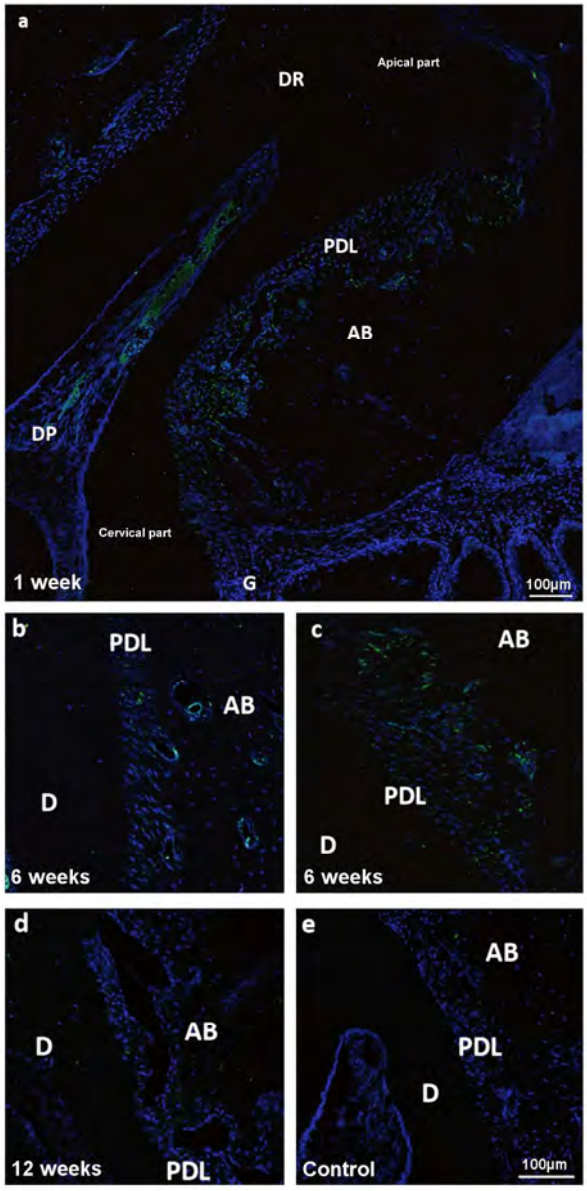


Fig. 1: Localization of the grafted GFP+ ASCs. Cells were tracked by immunofluorescence. (a) GFP+/ASC were identified in the experimental, periodontium-implanted site at 1 week, not only close to the wound bed near the cervical part but also toward the apical part of the PDL and surrounding the ligament and alveolar bone blood vessels. (b, c) GFP+/ASC localization surrounding PDL and alveolar bone blood vessels (b) and in the apical part of the PDL (c). (d) Undistinguishable GFP+/ASC in the grafted side at week 12. (e) Undistinguishable cells in vehicle-only treated control sites. AB: alveolar bone, D: dentin, DP: dental pulp, DR: dental root, G: gingiva, PDL: periodontal ligament. Bar: 100 μm
295x589mm (300 x 300 DPI)

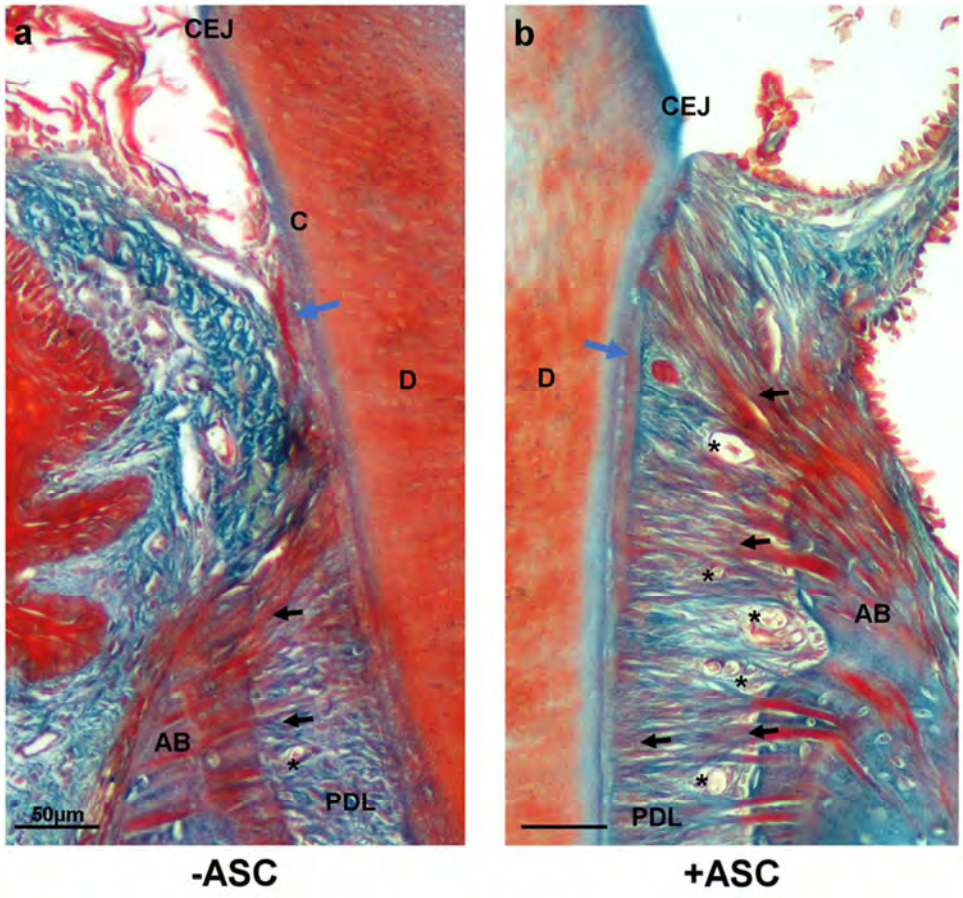


Fig. 2: ASC graft improved deep periodontium regeneration. Histological section of mouse deep periodontium 12 weeks after vehicle (a) or ASC (b) grafting. Cementum deposition (blue arrow), PDL fiber organization (black arrows), and number of vessels (*) had increased in the experimental, ASC-treated side compared to the control side. CEJ: Cemento-enamel junction. AB: alveolar bone, D: dentin, PDL: periodontal ligament. Bar: 50 µm
75x69mm (300 x 300 DPI)

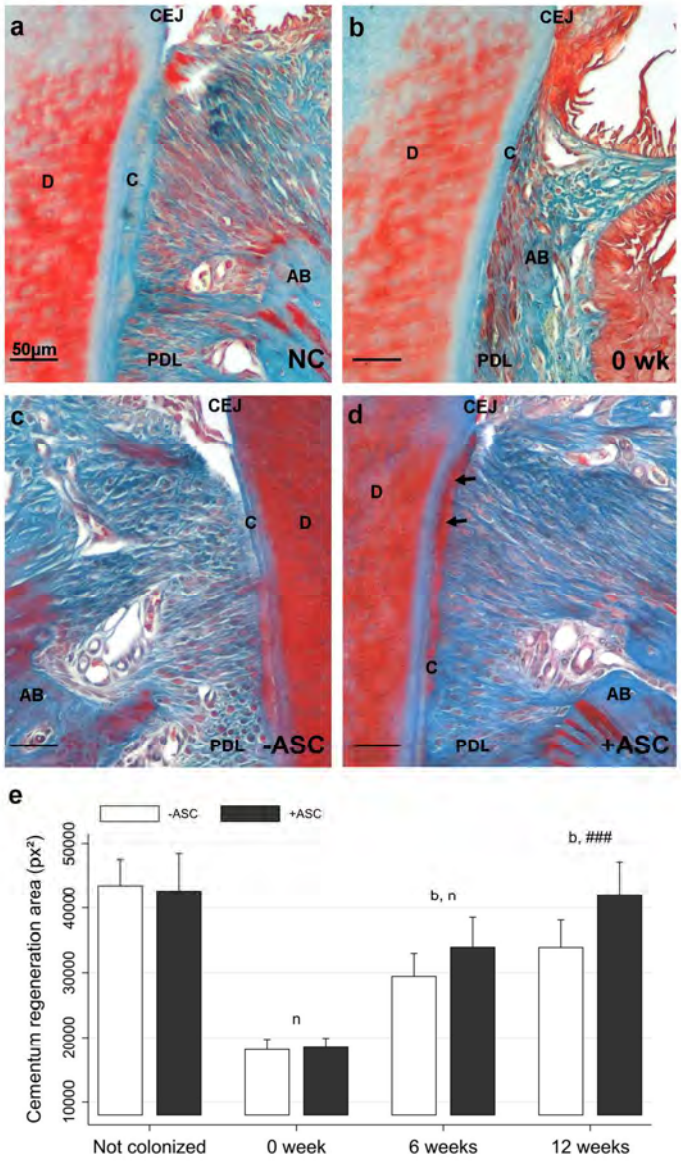


Fig. 3: ASC graft improved cementum regeneration. Histological sections of healthy (a), and diseased cementum before treatment (0 week (wk), (b)) and 12-week vehicle-only (c) or ASC (d) treated tissue. (e): Histomorphometry analysis of cementum deposition. The area of cementum was measured in square pixels in the control side (white bars) and the ASC grafted side (gray bars). 12 weeks after grafting, the cementum was rescued in the ASC treated side only. CEJ: Cemento-enamel junction. C: cementum, AB: alveolar bone, D: dentin, PDL: periodontal ligament. Bar: 50 µm. The "b" code indicates a significant difference in the treatment side between each time point and 0 week (baseline). The "n" code indicates a significant difference of the treatment side at each time point and not colonized. "#", "##" and "###" indicate a significant difference between treatment and control sides with $p<0.05$, $p<0.01$ and $p<0.001$ respectively. 195x336mm (300 x 300 DPI)

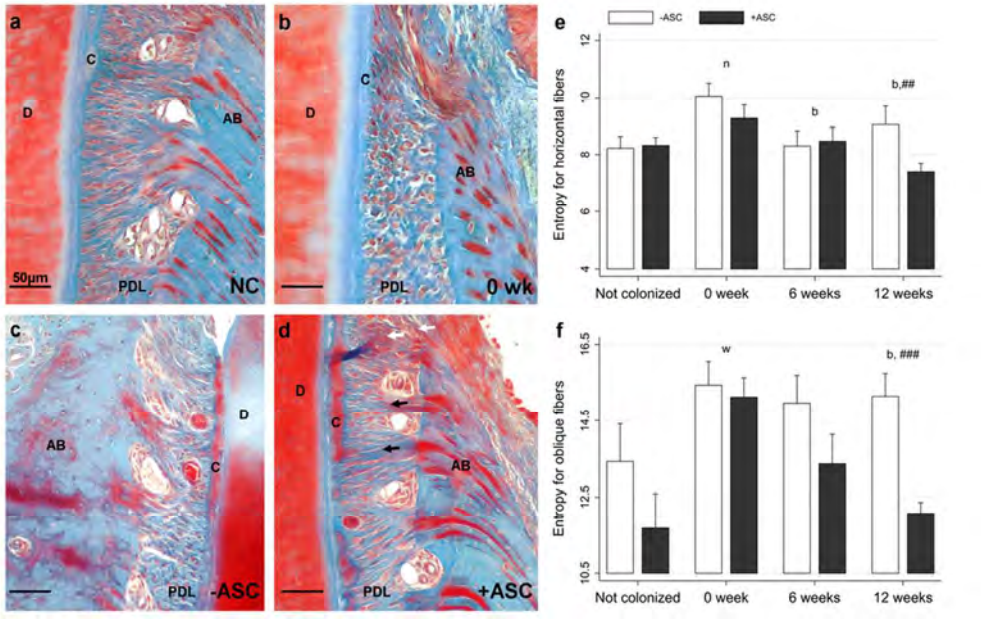


Fig. 4: ASC graft improved PDL fiber re-organization. (a - d): Histological sections of healthy (a) and diseased PDL fibers before treatment (0 week (wk), (b)) and 12-weeks vehicle (c) or ASC (d) treated. (e – f): Histomorphometry analysis of PDL. 12 weeks after treatment, the entropy calculated from the Hough transform was significantly lower in ASC grafted sites than controls, for both horizontal (e) and oblique (f) fibers, and close to that of healthy PDL. The "b" code indicates a significant difference in the treatment side between each time point and 0 week (baseline). The "n" code indicates a significant difference in the treatment side at each time point and not colonized. "#", "##" and "###" indicate a significant difference between treatment and control sides with $p<0.05$, $p<0.01$ and $p<0.001$ respectively. C: cementum, AB: alveolar bone, D: dentin, PDL: periodontal ligament. Bar: 50 µm.
92x57mm (300 x 300 DPI)

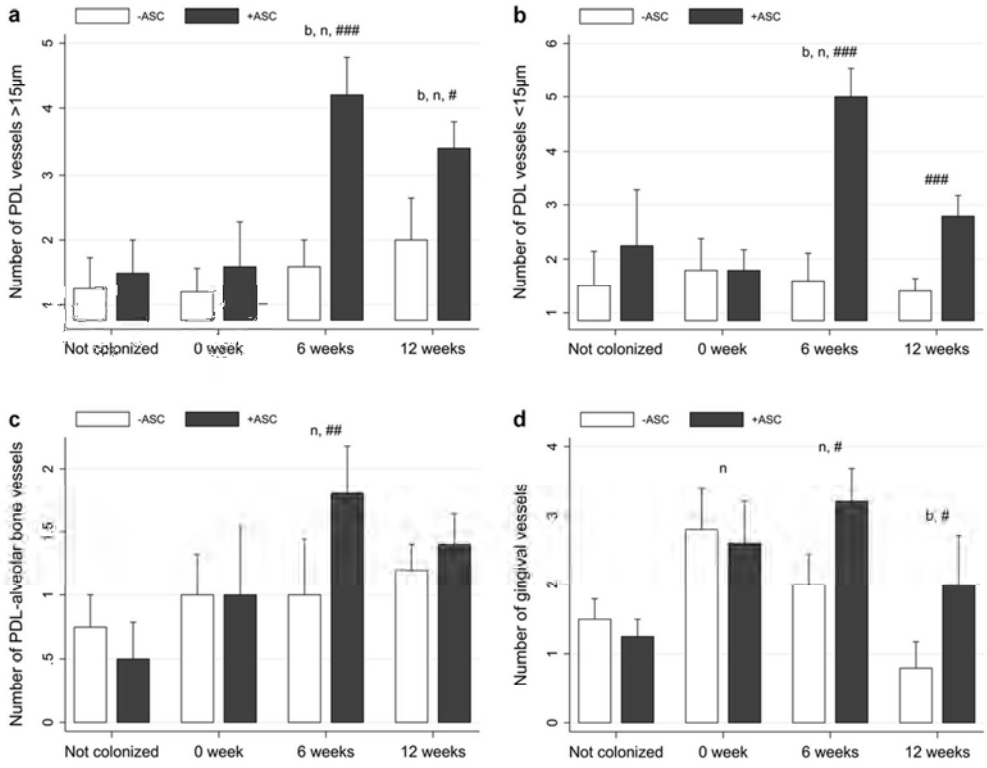


Fig. 5: ASC graft enhanced PDL neo-vascularization. (a - d) Six weeks after treatment, the number of >15µm diameter (a), <15µm diameter (b), alveolar bone (c) and gingival (d) PDL vessels was significantly higher in ASC-treated than vehicle-treated sites. The "b" code indicates a significant difference of the treatment side between each time point and 0 week (baseline). The "n" code indicated a significant difference of the treatment side at each time point and not colonized. "#", "##" and "###" indicate a significant difference between treatment and control sides with $p<0.05$, $p<0.01$ and $p<0.001$ respectively.
91x73mm (300 x 300 DPI)

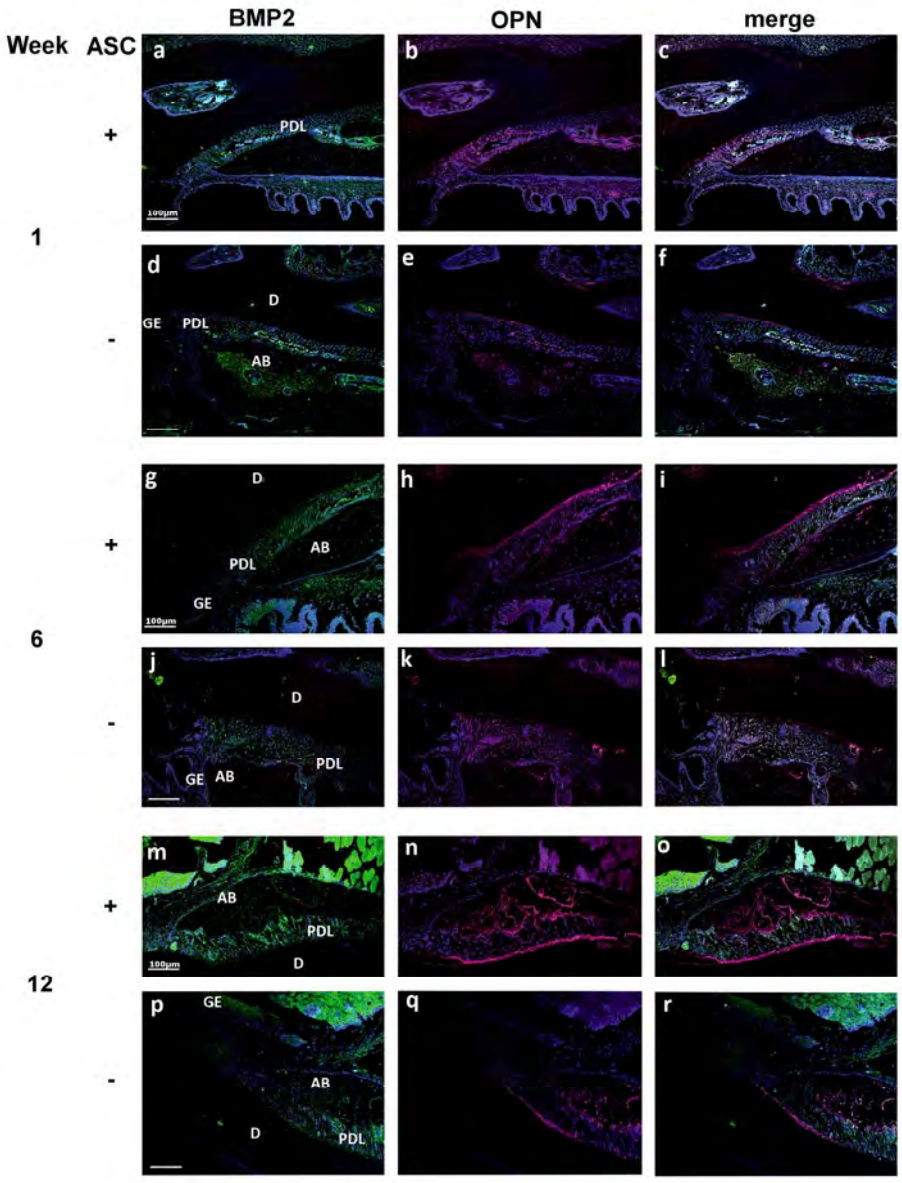


Fig. 6: Effect of ASC graft on BMP2 and OPN expression during periodontal healing. BMP2 (green, a, d, g, j, m, p) and OPN staining (magenta, b, e, h, k, n, q), and their co-localization (c, f, I, l, o, r) 1, 6 and 12 weeks after deep periodontium grafting +/- ASC. Six weeks after surgery, BMP-2 expression extended toward the apical part of the PDL in experimental side only. OPN expression underlined the cementum deposition and was clearly enhanced by ASC implantation compared to control. Cell nuclei in blue. AB: alveolar bone, D: dentin, GE: gingival epithelium, PDL: periodontal ligament. Bar: 100 µm
192x249mm (300 x 300 DPI)

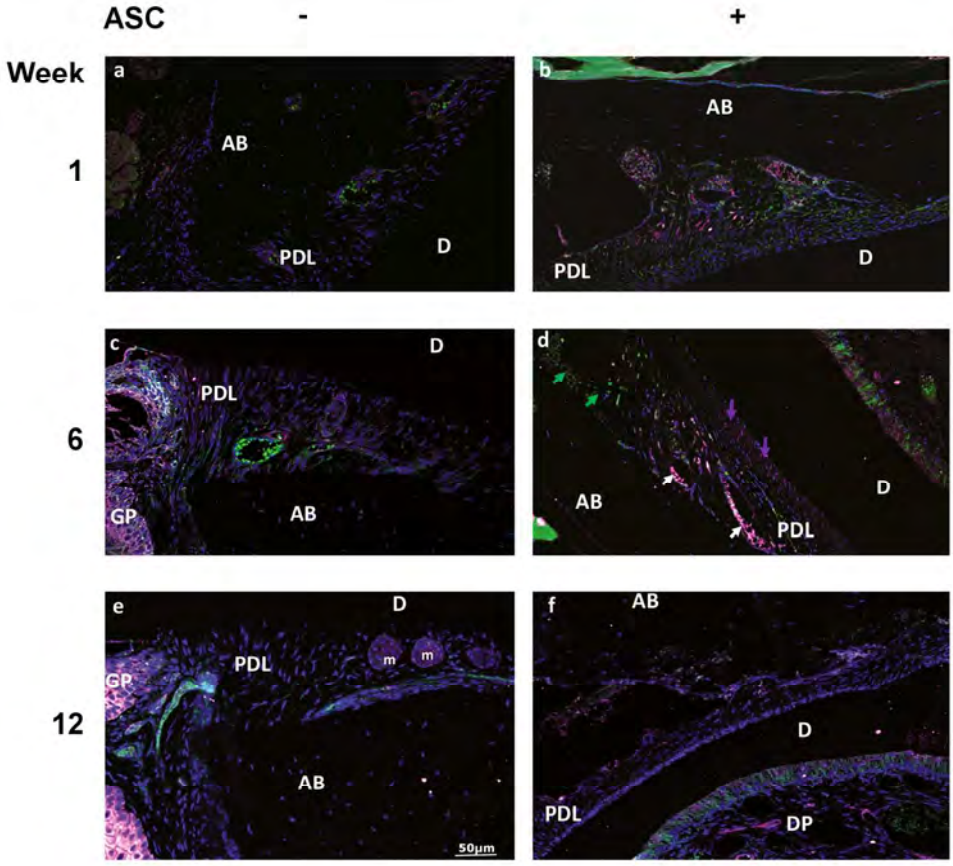
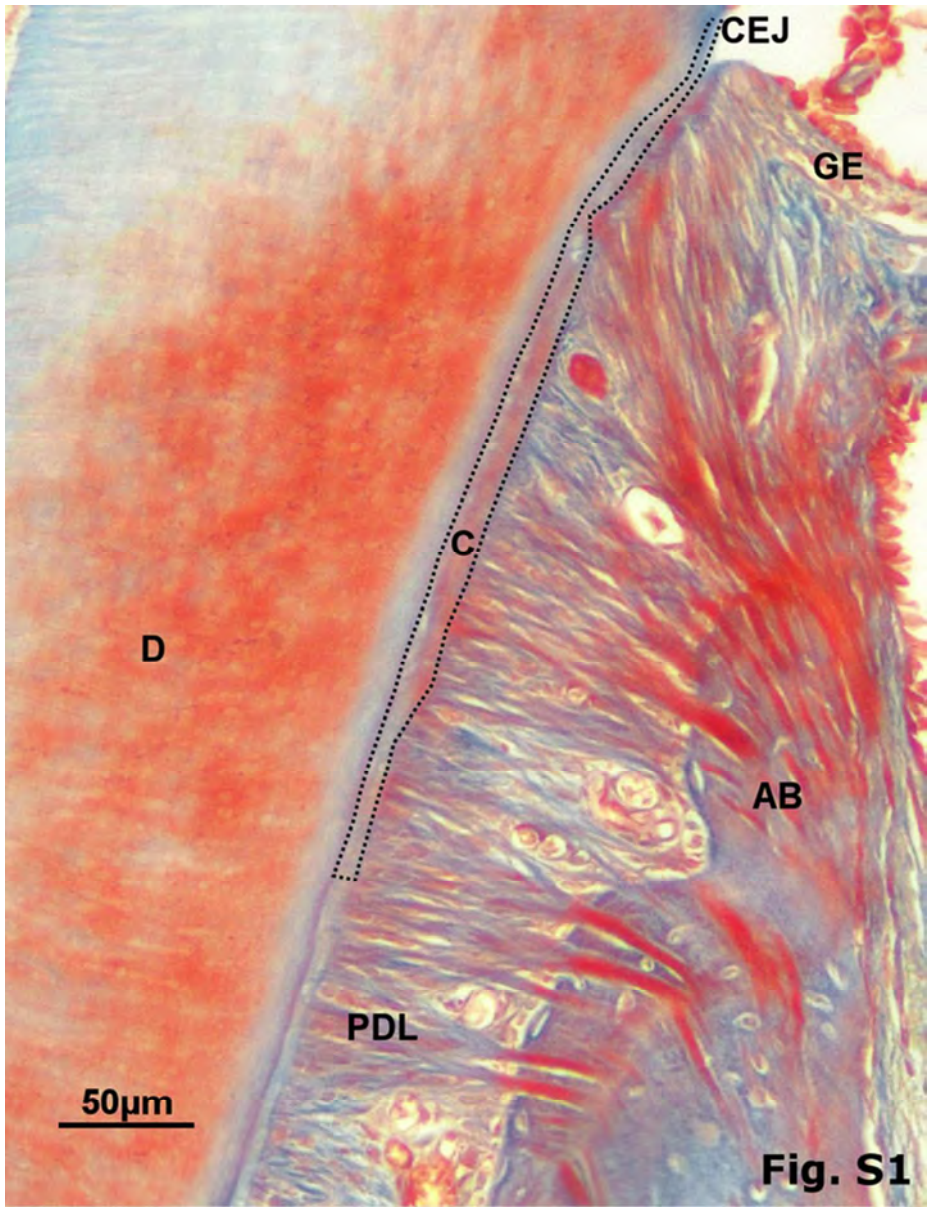


Fig. 7: ASC graft impacted the PDL expression of SCA-1 and CD146 during periodontal healing. Co-localization of SCA-1 (green) and CD146 (magenta) expression 1 (a, b), 6 (c, d) and 12 (e, f) weeks after deep periodontium grafting +/- ASC. After 6 weeks, ASC periodontium implantation only clearly promoted the emergence of SCA-1+/CD146- (green arrows) and SCA-1+/CD146+ (white arrows) populations in peri-vascular locations, and an SCA-1-/CD146+ (magenta arrows) subset indicating the cementum-lining cells. Cell nuclei in blue. AB: alveolar bone, D: dentin, GE: gingival epithelium, PDL: periodontal ligament. DP: dental pulp. Bar: 50 µm.
134x121mm (300 x 300 DPI)



56x73mm (300 x 300 DPI)

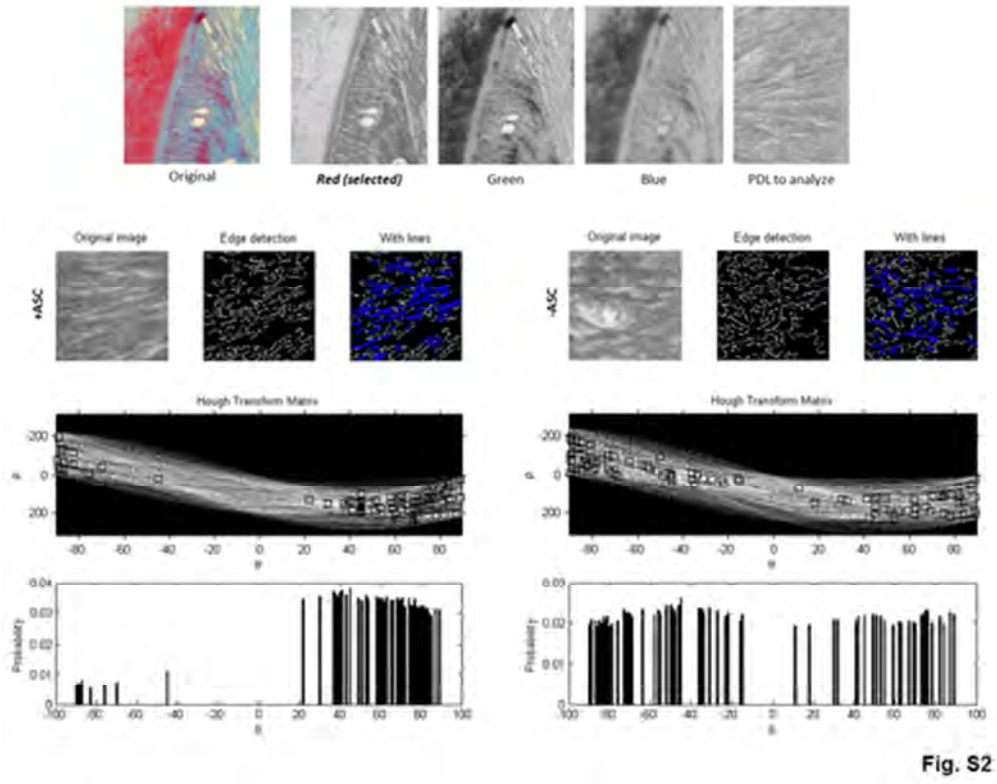


Fig. S2

43x34mm (300 x 300 DPI)

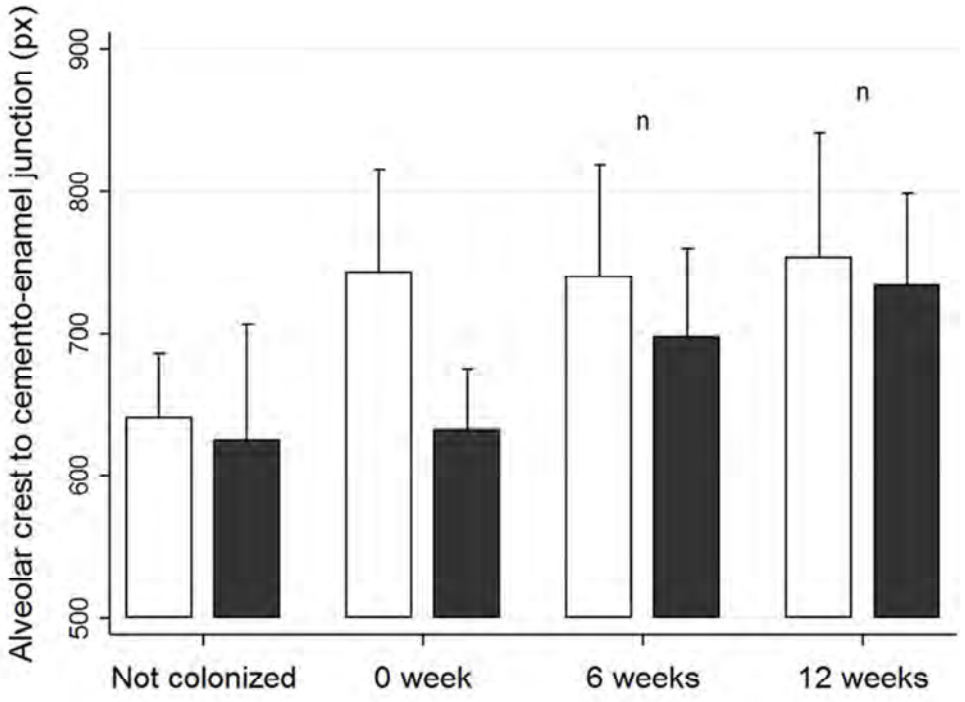


Fig. S3

65x52mm (300 x 300 DPI)

Antibodies		Supplier	Dosage (v:v)
Bone Morphoprotein (BMP-2) Goat anti-mouse	Primary	Santa Cruz technologies	1:50
Osteopontin (OPN) Rabbit anti-mouse	Primary	LF -175 Larry Fisher	1:400
SCA-1 Rat anti-mouse	Primary	BD Pharmingen	1:100
Rabbit anti-GFP Alexa 488	Primary	Invitrogen	1:75
CD146 Sheep anti-mouse	Primary	R&D systems	1:75
Swine anti-goat Alexa 488	Secondary	Invitrogen	1:200
Donkey anti-rabbit Alexa 594	Secondary	Invitrogen	1:150
Donkey anti-rat Alexa 594	Secondary	Invitrogen	1:200
Donkey anti-sheep Alexa 647	Secondary	Invitrogen	1:150
Goat anti-rat Alexa 488	Secondary	Invitrogen	1:200

Table S1.
List of used primary or secondary antibodies, with supplier and dosage

Original Research

January 15, 2016

Dear Editor,

We submit our article entitled "Periodontal tissue regeneration using syngeneic adipose-derived stromal cells in a mouse model" to be considered for publication in Stem Cells Translational Medicine.

In this study, we aimed to use adipose-derived mesenchymal stromal cells for periodontal regeneration in a new, relevant, bacteria-induced periodontitis model in mice.

Human periodontitis is a pervasive, chronic infectious disease characterized by the loss of both the soft and hard tissues supporting the teeth. By prevalence, periodontal disease is the second most infectious disease affecting mankind. Conventional periodontal therapy is currently associated with a high degree of variability in clinical outcome, regardless of therapeutic approach.
Thus, the regeneration of bone, cementum and an effective periodontal ligament, remains a challenge.

A previous systematic review of the literature we published in Stem Cells Translational Medicine demonstrated that periodontal regeneration by Mesenchymal Stromal Cells (MSC) was performed using poorly relevant defect models, and that data about graft of MSCs from adipose tissue (ASC) were sparse [1]. For many reasons related to safety in tissue sample processing, access to cell sources and availability, ASCs are expected to be a valuable source of cells.

In this study, we aimed to avoid such limitations by using a model with periodontal lesions induced by oral gavage with periopathogens, which led to periodontal defects relevant to human pathophysiology [1, 2]. We also used microscopic examination, immunofluorescence, and innovative bio-informatics histomorphometry methods to reveal deep periodontium changes.
After 12 weeks, cementum regeneration, the organization of PDL fibers, the number of periodontal ligament vessels were greater in experimental sites than in controls. Specific stromal cell subsets were recruited in the newly formed tissue in ASC-implanted periodontium only.

Our data so suggest that ASC grafting in diseased deep periodontium relevant for human pathology induces a significant improvement of the periodontal ligament microenvironment, leading to a recovery of tooth-supporting tissue homeostasis.

We hope you will judge favorably of this article to be published in your journal.
Thank you in advance for considering our submission.

Mathieu Lemaitre
Professor Philippe Kémoun

1. Monsarrat P, Vergnes JN, Nabet C et al. Concise Review: Mesenchymal Stromal Cells Used for Periodontal Regeneration: A Systematic Review. **Stem Cells Transl Med**. 2014.
2. Blasco-Baque V, Serino M, Vergnes JN et al. High-fat diet induces periodontitis in mice through lipopolysaccharides (LPS) receptor signaling: protective action of estrogens. **PLoS One**. 2012;7:e48220.

II.2.3.2 Principaux éléments de discussion et perspectives

Les ASCs favorisent la régénération cémentaire

La greffe d'ASCs a induit un dépôt de cément, et à sa surface de la protéine ostéopontine, significativement augmenté par rapport au contrôle. Ces données corroborent celles obtenues chez le chien pour la régénération de lésions inter radiculaires de grade III où la greffe d'ASCs a amélioré la quantité de cément formé (90). La greffe de CSMs du ligament parodontal chez le rat dans une lésion de type fenestration a également montré une augmentation d'ostéopontine (OPN). OPN est une protéine non-collagénique qui joue un rôle dans la cohésion entre la phase minérale et organique en se liant au collagène matriciel et au calcium, permettant l'initiation de la formation d'hydroxyapatite sur les fibres (91-93). OPN est également impliquée lors de la phase inflammatoire en favorisant le recrutement des macrophages et le remodelage osseux (92). La superfamille des TGFβ a été montrée comme inductrice de la différenciation des cellules précurseurs en cémentoblastes (94) ; ainsi, par ces sécrétions d'OPN (95) et de TGFβ (96), les ASCs pourraient promouvoir la régénération cémentaire.

Les ASCs favorisent un ligament parodontal mieux organisé

La littérature était insuffisante quant à la quantification des paramètres architecturaux du ligament parodontal. Pourtant, un ligament bien structuré avec des fibres bien orientées et intègres, est garant d'une répartition homogène des forces masticatoires, d'un remodelage harmonieux de l'os alvéolaire et d'une nutrition adéquate du cément (97). Dans ce modèle, même s'il n'existe pas de différence significative au niveau de l'épaisseur du ligament, les paramètres mathématiques ont démontré que les ASCs stimulaient la régénération de fibres ligamentaires mieux organisées, avec une plus grande densité et intégrité (diminution de l'HT pour les fibres horizontales et obliques du côté ASCs comparé au côté contrôle). Lors de futures études, l'utilisation de la microscopie bi-photonique, serait particulièrement indiquée pour aller plus en détail dans ces analyses. Comme cela a été suggéré dans un modèle de lésion de la coiffe des rotateurs chez le rat, la greffe d'ASCs permettrait de rétablir un signal TGFβ1 et TGFβ3 adéquat, ainsi qu'un ratio favorablement augmenté de collagène type I/ collagène type III (96).

Il a été aussi précédemment montré que des protéines appartenant à la superfamille du TGFβ, les BMPs, modulent la fibrogenèse sous le contrôle du CTGF, un facteur mitogénique et chimiotactique impliqué dans la prolifération cellulaire et la synthèse matricielle (98). En outre,

au cours de la morphogenèse parodontale, il a été souligné le rôle primordial des BMPs, qui sont exprimées dans le desmodonte selon une séquence spatiale et temporelle précise (99). Les BMP2 et BMP7 sont de puissants facteurs ostéo-inducteurs (100). Ils sont capables d'induire le recrutement et la stimulation de CSMs vers la différenciation en cellules spécialisées des matrices calcafines (101), et pourraient donc participer à la différenciation des précurseurs desmodontaux en cémentoblastes et ostéoblastes au cours de la régénération des poches parodontales. D'ailleurs, il a été rapporté que les ASC pouvaient stimuler la néoformation de tissu osseux en présence de BMP 2 et 7 (102). On peut noter également que la différenciation ostéogénique d'ASC est associée à une activation des voies de signalisation de la BMP6 (103).

Les ASCs favorisent la néo vascularisation

Nous avons montré une augmentation significative du nombre de vaisseaux dans le ligament du côté greffé par rapport au côté contrôle. Les CSMs endogènes et les ASCs sont situées majoritairement autour des vaisseaux. Ces données sont en accord avec celles obtenues dans un modèle murin de cicatrisation cutanée (104). Il est possible d'une part que des ASCs acquièrent des caractéristiques de péricytes (105), et d'autre part que des péricytes acquièrent certaines fonctionnalités de CSMs (106, 107). Le rôle de ces péricytes est ainsi de stabiliser les cellules endothéliales pour améliorer la formation et la fonction du réseau vasculaire (105).

Les ASCs favoriseraient la néo vascularisation par une transdifferenciation en cellules endothéliales CD31+ (104) mais également par leur activité de sécrétion paracrine ; en effet, les facteurs VEGF (régulateur clef de l'angiogenèse), HGF et FGF sont impliqués en tant qu'agents angiogéniques et mitogéniques (104, 108, 109). D'autres mécanismes sont probablement en jeu. Les ASCs ont la capacité d'activer des métalloprotéases matricielles, ce qui permet d'amplifier le signal VEGF (110) par la dissociation du complexe VEGF/CTGF. Les ASCs ont également la capacité de sécréter de la BMP-2 (de la superfamille des TGFβ (102)), qui a une action à la fois d'osteoinduction (101) mais également un effet chimiotactique pour les cellules endothéliales (111).

Les ASCs favorisent le recrutement de cellules progénitrices mésenchymateuses

Nous avons montré par immunofluorescence, la diminution des cellules greffées GFP+ au fur et à mesure du temps, avec une augmentation par rapport au contrôle de deux populations

cellulaires distinctes : une population CD146+/SCA-1+ située au niveau peri-vasculaire, et une population CD146+/SCA-1- localisée en périphérie du cément. Nous posons donc l'hypothèse que les ASCs favoriseraient le recrutement des progéniteurs parodontaux endogènes (106, 107). Une partie de ces progéniteurs se différencierait par la suite en cémentoblastes, d'où la localisation sous la couche cémentaire de la population CD146+/SCA-1- qui correspondrait à un pool cellulaire pré-cémentoblastique.

Nous faisons l'hypothèse que le signal chimiotactique augmenté après la greffe d'ASCs (59) pourrait être une des raisons de cette mobilisation des cellules progénitrices (potentiellement locales, locorégionales ou circulantes).

Vers le gros animal

Nous n'avons pas mis en évidence de néoformation osseuse significative concernant la mesure de la distance crête osseuse alvéolaire – jonction amélo-cémentaire. L'anatomie supra-alvéolaire des lésions osseuses (85) pourrait être une explication de cette absence de régénération osseuse verticale. Ces résultats sont à rapprocher de ceux de Young *et al* qui n'a pas réussi non plus à démontrer de régénération osseuse significative dans un modèle similaire au notre (87). La confirmation dans un modèle de gros animal, le modèle chien en particulier, est nécessaire. Dans ce modèle, le contexte inflammatoire n'a pas été étudié ; cela sera nécessaire dans le modèle canin.

II.3 Effet antibactérien des CSMs

II.3.1 Article 7: « Broad spectrum antibacterial effects of human adipose-derived stromal cells"

Le travail présenté dans cette partie fait l'objet d'un article en cours d'écriture.

II.3.2 Introduction

Comme nous l'avons envisagé dans la première partie, les CSMs sont utilisées de manière très large en médecine régénérative. Des infections polymicrobiennes sont également mises en jeu lors de ces pathologies comme les brulures, les plaies chroniques chez les patients diabétiques, les infections chroniques des poumons ou les parodontites (112).

Les plaies chroniques des pieds sont des infections fréquentes chez les patients diabétiques, causées par des ulcérations de la peau ou des lésions traumatiques. La progression de ces pathologies est favorisée par des perturbations au niveau de la perception neurosensorielle et des fonctions immunes et cardio-vasculaires (112). Le microenvironnement de la plaie est perturbé par un infiltrat inflammatoire et une colonisation bactérienne (113). Les plaies sont colonisées par des bactéries aérobies gram-positive, particulièrement *Staphylococci spp* les plus souvent retrouvées (114). Ces infections mettent également en jeu des bactéries anaérobies, avec des infections mixtes dans 50% des cas (112). *Fusobacterium spp* et *Porphyromonas spp* peuvent être retrouvées par écouvillonnage. Ces bactéries peuvent diffuser profondément dans les tissus, atteindre l'os et altérer le pronostic (114). La qualité de vie des patients est de fait fortement affectée puisque 20% des patients hospitalisés subissent une amputation, et la survie à 5 ans équivaut à certains cancers (115). Les traitements actuels des formes avancées se basent donc sur un débridement des tissus nécrotiques et une tentative de revascularisation (114). Dans ce cadre-là, l'utilisation d'ASCs et de ses propriétés pro-angiogéniques (soutien local de la néovascularisation avec la sécrétion de facteurs pro-angiogéniques et la stabilisation des néovaisseaux, transdifferenciation éventuelle en cellules de type endothéliale ou péricytaire) donne des résultats très prometteurs (104, 116-120).

L'apport de CSMs, et plus particulièrement d'ASCs se ferait donc dans des environnements où il reste un contingent inflammatoire et bactérien. Comme des analyses transcriptomiques suggèrent que les macrophages et les ASCs partagent l'expression d'un grand nombre de gènes (121, 122) et que les ASCs ont été montrés capables d'internaliser le champignon *Candida*

albicans et d'avoir une activité microbicide (123), nous avons tenté d'évaluer le comportement des ASCs en contact avec des bactéries gram-négatives et gram-positives, dont des paro-pathogènes.

II.3.3 Matériel et méthodes

Culture bactérienne

La Table II-1 présente toutes les souches testées, provenant de la collection CIP ou de la collection ATCC, ainsi que la méthode de culture employée. Pour réaliser l'infection des cellules, les bactéries ont été cultivées dans un bouillon de Wilkins Chalgren toute la nuit. Les bactéries ont été centrifugées durant 10 minutes à 1800g, puis lavées dans du PBS. La densité optique (DO) a été mesurée à 600 nm, et grâce à une formule de conversion DO_{600nm} vers nombre de bactéries préalablement établie, les souches ont été diluées de manière appropriée dans un milieu α-MEM contenant 10% de sérum de veau fœtal (SVF) décomplémenté. La dose initiale de bactéries a été calculée de manière à obtenir environ 6 à $6.5x10^6$ unités formant des colonies (UFC) après 6 heures d'incubation à 37°C.

Souche bactérienne	Référence	PEROX	CAT	SOD	Condition de culture
Lactobacillus casei	CIP 103137	Non	Non	Non	Gélose Cœur/Cerveau – Aérobie
Streptococcus sanguinis	CIP 55128	Oui	Non	Oui	Gélose TCS avec 10% sang de mouton défibriné, 5µg/ml d'hémine et 1µg/ml de ménadione – Aérobie
Staphylococcus aureus	ATCC 888807	Oui	Oui	Oui	Gélose Cœur/Cerveau – Aérobie
Escherichia coli	ATCC 25922	Oui	Oui	Oui	Gélose Cœur/Cerveau – Aérobie
Enterococcus faecalis	ATCC 29212	Oui	Oui	Oui	Gélose Cœur/Cerveau – Anaérobie
Porphyromonas gingivalis	ATCC 33277	Oui	Non	Oui	Gélose TCS avec 10% sang de mouton défibriné, 5µg/ml d'hémine et 1µg/ml de ménadione - Anaérobie
Fusobacterium nucleatum	ATCC 25586	Non	Non	Non	
Prevotella intermedia	CIP 103607	Oui	Non	Non	

Table II-1 : Caractéristiques des souches. Cette table présente les souches bactériennes utilisées dans cette étude, leur collection d'appartenance (CIP ou ATCC). L'expression de la protéine peroxydase (PEROX), catalase (CAT), superoxide dismutase (SOD) est fournie selon les données d'UniprotKB (http://www.uniprot.org/uniprot/) en respectant la classification du Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB). Les conditions de culture, type de gélose et atmosphère, sont données. Les incubations ont été réalisées à 37°C ; pour l'anaérobiose, les souches ont été placées dans des jarres *ad hoc* (GENbox anaer, Biomérieux).

Culture des ASCs

Des prélèvements de tissu adipeux humain sous-cutané abdominal ont été utilisés comme précédemment décrit afin d'isoler les ASCs (124). Ces prélèvements sont issus des dermolipectomies abdominales réalisées par le service de Chirurgie plastique et des brûlés du CHU de Toulouse (Pr Grolleau-Raoux). Le consentement des patients a bien sûr été obtenu pour l'exploitation du tissu. L'âge, le sexe, taille, poids et IMC des patients sont détaillés dans la Table II-2. Il n'y a pas de différence significative d'âge entre les patients des catégories d'IMC poids normal, surpoids ou obésité.

Le tissu a été digéré dans une solution de PBS contenant 2% d'albumine sérique bovine et 2mg/ml de collagénase de type I (Sigma-Aldrich) pendant 45 minutes à 37°C sous agitation linéaire constante. L'ensemble a été filtré à 25µm, puis centrifugé à 800g pendant 8 minutes pour enlever les adipocytes matures. Les globules rouges ont été lysés dans une solution tampon contenant 140nM de NH_4Cl et 20mM de Tris pendant 5 minutes à 4°C. Les cellules ont été centrifugées à 600g pendant 5 minutes et la fraction stromale vasculaire (SVF) ensemencée à 4000 cellules/cm² dans un α-MEM avec 0.25 µg/ml d'amphotéricine B, 100µg/ml de streptomycine, 100 UI/ml de pénicilline, en atmosphère 5% CO2. Le jour suivant, les cellules ont été rincées en PBS puis maintenues dans un milieu de culture exempt d'antibiotique et d'antifongique. Les cellules ont été utilisées du passage 1 à 2.

	IMC < 25	**25 ≤ IMC < 30**	**IMC ≥ 30**	**Tous**
Nombre	18	19	5	42
Age	40.7 ± 8.4	41.4 ± 9.4	40.9 ± 19.6	41.1 ± 10.3
Sexe (H/F)	1/17	1/18	0/5	2/40
Taille	168.8 ± 8.0	162.7 ± 7.2	159.4 ± 7.9	165 ± 8.26
Poids	66.8 ± 6.6	71.6 ± 6.8	80 ± 8.5	70.5 ± 8.0
IMC	23.4 ± 1.2	26.9 ± 1.5	31.4 ± 0.9	26.0 ± 2.9

Table II-2 : Caractéristiques des donneurs employés dans cette étude, en moyenne ± SD

Evaluation de la viabilité bactérienne sur gélose

Pour énumérer les colonies bactériennes, une méthode par microgouttelettes 6x6 a été utilisée (125). Les échantillons ont été dilués de manière sériée en utilisant une pipette multicanaux dans des plaques de 96 puits, et des gouttelettes de 10µl ont été déposées par pipetage inversé sur la gélose. Après un temps d'incubation variable selon les souches, le nombre de colonies bactériennes a été compté en base log10 selon le facteur de dilution.

Evaluation de l'intégrité membranaire

Après incubation, le milieu a été agité plusieurs fois et 50µl collecté pour être marqué dans une solution de 200µl de PBS contenant de l'iodure de propidium (Sigma-Aldrich) et du Syto-62© (Life technologies) à concentration finale de respectivement 20 µM et 1 µM. Pour la mesure du potentiel membranaire, le milieu a été marqué au 3,3'-Dihexyloxacarbocyanine Iodide (DioC6(3), 1 µm, Sigma-Aldrich) (126)). Toutes les expérimentations ont été analysées par cytométrie en flux au FACSCalibur (BD Biosciences).

Microscopie électronique à balayage

Les cellules ont été cultivées sur lamelle en plastique de 18mm x 18mm jusqu'à 60-70% confluence, puis infectées pendant 6 heures avec des bactéries. Le milieu contenant les bactéries a été filtré à 0.22µm pour les concentrer avant traitement.

Les cellules ou les bactéries ont été fixées en tampon Sorensen 2% glutaraldehyde pendant au moins 4 heures à 4°C. Après 12 heures de lavage dans un tampon de 0.1M cacodylate de sodium, les échantillons ont été déshydratés en éthanol croissant, séchés grâce à l'EMSCOPE CPD 750 (point critique), et recouverts d'une fine couche de platine de 2nm. Les échantillons ont été observés à l'ESEM Quanta 250 FEG (FEI, USA) à 5kV (127).

Microscopie électronique à transmission

Les échantillons ont été fixés comme précédemment décrit, puis post-fixés dans un tampon Sorensen à 1% d'OsO_4 pendant 1 heure suivi d'une déshydratation à l'éthanol et à l'oxyde de propylène, et enfin un montage en résine époxy (Epon 812). Des sections ultra-minces de 70nm ont été montées sur des grilles de cuivre recouvertes de collodion et contre-colorées à l'acétate d'uranyle 3% dans un tampon 50% éthanol, 8.5% citrate de plomb. L'observation a été réalisée sur un HT 7700 Hitachi à 80kV (127).

Evaluation du contact nécessaire entre bactéries et cellules

Après 6 heures d'incubation, les milieux de culture (avec ou sans cellules, avec ou sans bactéries) ont été collectés et filtrés à 0.22µm. Les milieux conditionnés (avec bactéries) ou les

milieux naïfs (sans bactéries) ont été congelés à -20°C pour éliminer les résidus bactériens. Des échantillons de 90µl de milieu conditionné ont été transférés dans des plaques de 96 puits, et infectés à nouveau avec 10µl de milieu de culture pour obtenir une concentration suffisante de bactéries.

Des plaques 12 puits contenant des inserts PET de 0.4µm (Merck-Millipore, Darmstadt, Allemagne) ont été utilisées pour les analyses en transwell. La Figure II-18A résume les différentes conditions testées. Soit la partie interne soit externe du transwell a été infectée, avec ou sans les ASC. Lorsqu'on compare les parties internes, avec et sans ASC, on obtient l'évaluation de l'effet indirect. Lorsqu'on compare les parties externes, avec et sans ASC, on obtient l'évaluation de l'effet direct. Après 6 heures d'incubation, la perméabilité membranaire a été évaluée par cytométrie en flux comme décrit précédemment.

Mesure des radicaux libres oxygénés (RLO)

Le diacetoxymethyl ester 6-carboxy-2', 7'-dichlorodihydrofluorescein diacetate (H2DCFDA, Life technologies) a été utilisé comme marqueur de RLO. Ce marqueur est non fluorescent dans sa forme réduite, facilement perméable à la membrane. Le groupe fonctionnel chlorométhyl se lie aux thiols cellulaires, retenant ainsi le marqueur au sein de la cellule. L'oxydation convertit ce marqueur en marqueur fluorescent. Les cellules à 80% de confluence ont été marquées par 4µm de H2DCFDA pendant 30 minutes dans du PBS à 37°C. Les cellules sont ensuite incubées dans du milieu de culture durant 30 minutes avant d'être exposées à une solution bactérienne ou à un contrôle. Les bactéries ont été ajoutées à 1 :100 MOI durant 45 minutes, ou à différents temps. Pour les inhibiteurs, ceux-ci ont été ajoutés 30 minutes avant l'infection, puis durant la totalité de la période d'incubation. Les cellules ont ensuite été trypsinisées et la fluorescence verte mesurée immédiatement par cytométrie en flux. Les inhibiteurs suivants ont également été testés : l'antioxidant N-acetyl cysteine (4mM, Sigma-Aldrich), l'inhibiteur de la NADPH (apocynin, 100µM, Sigma-Aldrich), le mimétique de la SOD, piégeur de peroxynitrite scavenger avec une activité catalase-like (MnTBAP, 50µM, Calbiochem, Merck Millipore), l'inhibiteur de NO synthase (L-NG-Nitroarginine Methyl Ester ou L-NAME, 100µM, Sigma-Aldrich) et l'inhibiteur de polymérisation de l'actine (cytochalasin D, 0.4µM, Sigma-Aldrich).

Expérimentation de phagocytose, visualisation en microscopie confocale

Avant infection, les bactéries ont été marquées au carboxyfluorescein succinimidyl ester (CFSE, Sigma-Aldrich, Lyon, France). Après centrifugation, les bactéries ont été incubées pendant 1 heure dans du PBS contenant 20µM de CFSE à température ambiante dans le noir. Après double lavage au PBS, les bactéries ont été à nouveau incubées 30 minutes en PBS puis rincées 2 fois. La quantification a été réalisée en cytométrie en flux, en utilisant des microbilles (AbC® Anti-Mouse Bead Kit, Invitrogen) comme référence.

Pour l'évaluation de la phagocytose, des chambres 8 puits PCA (Sarstedt, Marnay, France) ont été coatées avec une solution de gélatine à 1%. Les cellules ont été apportées et mises en culture pour obtenir une confluence d'environ 70%. L'infection par *Fn* et *Sg* a été faite à 1:100 MOI pendant 1 heure. Les cellules ont été fixées en paraformaldehyde à 3.7% pendant 15 minutes, puis perméabilisées avec 0.3% de Triton X100 pendant 20 minutes. Le blocage s'est fait avec du PBS+2% BSA durant 30 minutes à température ambiante. L'anticorps primaire souris anti-LAMP-1 (H4A3, DSHB) a été incubé à 1/100 v/v pendant 1 heure et lavé 3 fois 5 minutes avec du PBS. Chez les contrôles négatifs, les anticorps primaires ont été remplacés par l'isotype souris IgG1. Les anticorps secondaires, anti-souris Dylight 650 (1/200 v/v), ont été incubés pendant 1 heure et lavés 3 fois 5 minutes en PBS. Les noyaux ont été contre-colorés en Hoechst 33342 (5mg/mL) pendant 30 minutes. Après lavage, les lames ont été montées avec du milieu de montage pour fluorescence de chez Dako (Dako, Glostrup, Danemark). L'observation de la fluorescence a été réalisée par microscopie confocale (ApoTome, Zeiss).

Pour les analyses en time-lapse, des chambres 8 puits PCA ont été utilisées pour cultiver les ASCs à 70% confluence. Les cellules ont été marquées avec du CellTrace® Far Red (Life technologies, Saint-Aubin, France) à 2µM, puis infectées par des *Sg* préalablement marqués au CFSE (1 :100 MOI). Les cellules ont été monitorées pendant 14 heures en spinning disk (Nikon, Champigny-sur-Marne, France).

Expérimentation de phagocytose, évaluation de la diminution du pH

Les bactéries ont été marquées avec 1mM de pHrodo® Green STP Ester (Molecular Probes Life technologies) selon les recommandations du fabricant. Le témoin négatif (isotype) consistait à marquer du bouillon de culture non inoculé. Les cellules ont été cultivées jusqu'à 80% de confluence sur des plaques 6 puits, puis infectées à une MOI de 1:100 pendant 1 heure.

Pour démontrer la phagocytose, les cellules ont été comparées avec des cellules préalablement traitées à la cytochalasin D pendant 45 minutes avant et durant le temps d'incubation. Après trypsinisation, les cellules ont été récupérées en PBS+2% BSA pour analyse par cytométrie en flux (pourcentage et intensité de fluorescence verte).

qRT-PCR

Les cellules ont été lysées après avoir été exposées ou non pendant 6 heures à *Fn*, *Sg* ou *Sa*. Les ARN ont été récupérés en utilisant un kit spécifique (Rneasy; Qiagen, Courtaboeuf, France). La liste des sondes oligonucléotides utilisées dans cette étude est décrite en Table II-3.

Gene	5' – Sens	5' - Anti sens
TLR2	AGCAGGATCCAAAGGAGACC	GGCATTGTCCAGTGCTTCAA
TLR3	GGAAAGGCTAGCAGTCATCC	GTGGTGGAGGATGCACACAG
TLR4	TCCTGCGTGAGACCAGAAAG	TTGAGAAGGGGAGGTTGTCG
HBD1	CTGAAATCCTGGGTGTTGCCT	CCAAGGCCTGTGAGAAAGTT
HBD3	TGGTGCCTGTTCCAGGTCAT	TTCCTCCTTTGGAAGGCAGC
LL37	CTCGGATGCTAACCTCT	CATACACCGCTTCACC
MCP1	GTCTCTGCCGCCCTTCTGTGC	CAGCAGGTGACTGGGGCATTG
IFNβ	CCTGTGGCAATTGAATGGG	GGCGTCCTCCTTCTGGAAC
CXCL2	GGGCAGAAAGCTTGTCTCAACCCCG	TGTAAGGGCAGGGCCTCCTTCAG
IL1β	CCACAGACCTTCCAGGAGAATG	GTGCAGTTCAGTGATCGTACAGG
IL6	TCCACAAGCGCCTTCGGTCC	GCAGGGAAGGCAGCAGGCAA
IL33	GCCTGTCAACAGCAGTCTACTG	TGTGCTTAGAGAAGCAAGATACTC
TNFα	CAGGCGCCACCACGCTCTTC	CTGGGGAACTCTTCCCTCTGGGG
IL10	GCCGTGGAGCAGGTGAAG	TGGCTTTGTAGATGCCTTTCTCT

Table II-3 : Liste des primers utilisés

Analyses statistiques

La plupart des tests statistiques ont été réalisés en non-paramétrique et en série appariée. En effet, pour chaque souche bactérienne, le nombre d'UFCs était tellement important (6 à 7 logarithmes base 10 final) qu'il était possible de considérer la population bactérienne unique et homogène, comme s'il s'agissait d'un même donneur. Les données sont exprimées en moyenne ± erreur standard au niveau graphique et en moyenne ± écart-type au niveau des tableaux de données. Pour la réalisation des modèles multivariés, un modèle mixte a été mis en place dont la partie aléatoire se composait de l'identifiant de l'expérimentation niché dans l'identifiant du donneur de cellules, et la partie fixe des autres covariables. Les statistiques et les représentations

graphiques ont été générées en utilisant Stata 13.0 (StataCorp, Tx, USA). Pour la modélisation de la croissance bactérienne, le modèle de Gompertz a été utilisé via le logiciel R pour estimer les paramètres.

II.3.4 Résultats

Les ASCs possèdent un effet antibactérien rapide

Une diminution significative du nombre d'UFCs a été retrouvée pour les quatre souches bactériennes testées (*Lc*, *Ec*, *Sg* et *Sa*) une fois mises en contact avec les cellules. Cet effet était maximal à 6 heures bien qu'une tendance pouvait être vue à 4 et 9 heures (Figure II-6). La proportion de bactéries IP positives en cytométrie en flux suggérait un nombre maximal de bactéries perméabilisées entre 24.000 et 48.000 cellules par puits d'une plaque 12 puits (Figure II-7). Une confluence de 80% avec un temps d'incubation de 6 heures ont donc été choisis.

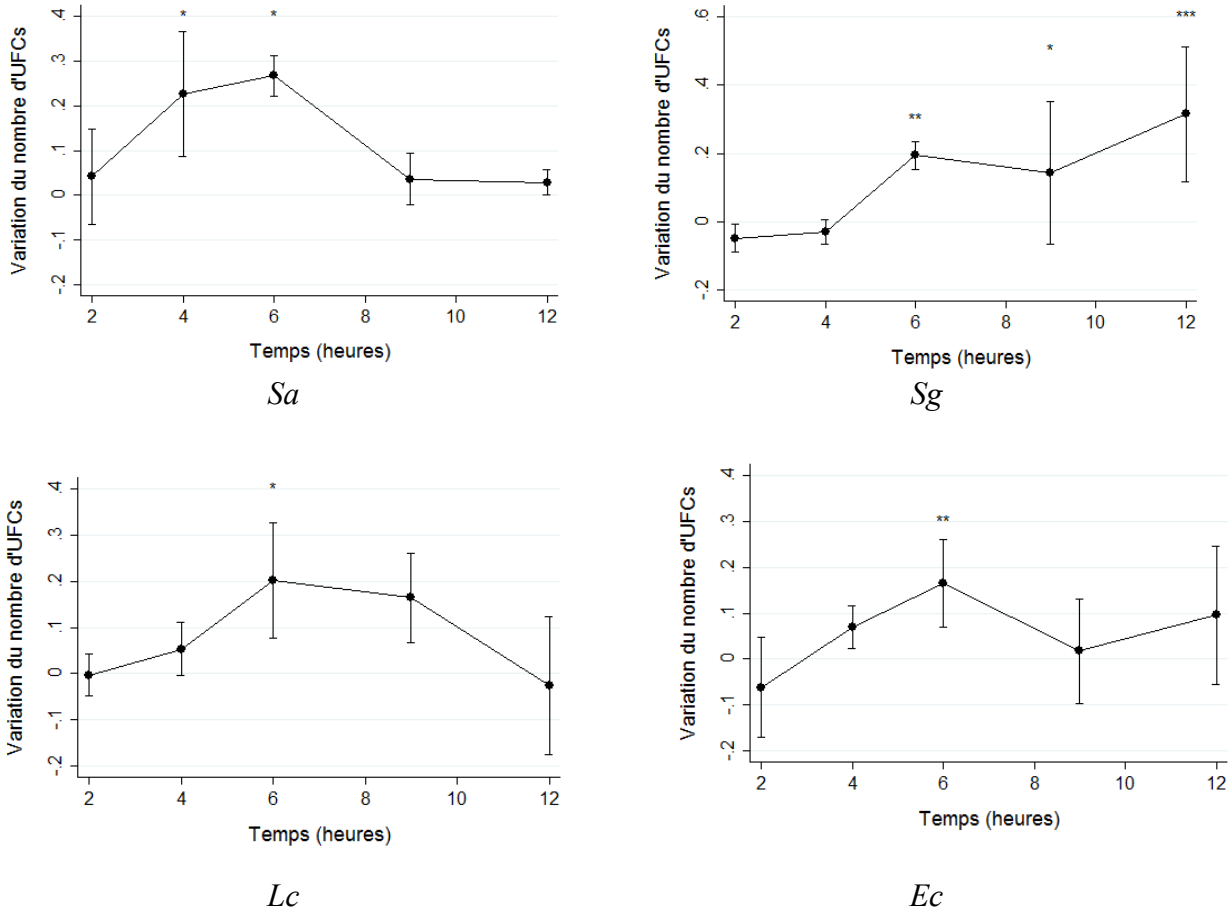


Figure II-6 : Les ASCs ont une inhibition antibactérienne maximale à 6 heures. La différence entre le nombre d'UFCs sans et avec contact avec les ASCs a été mesurée à différents temps (N=6).

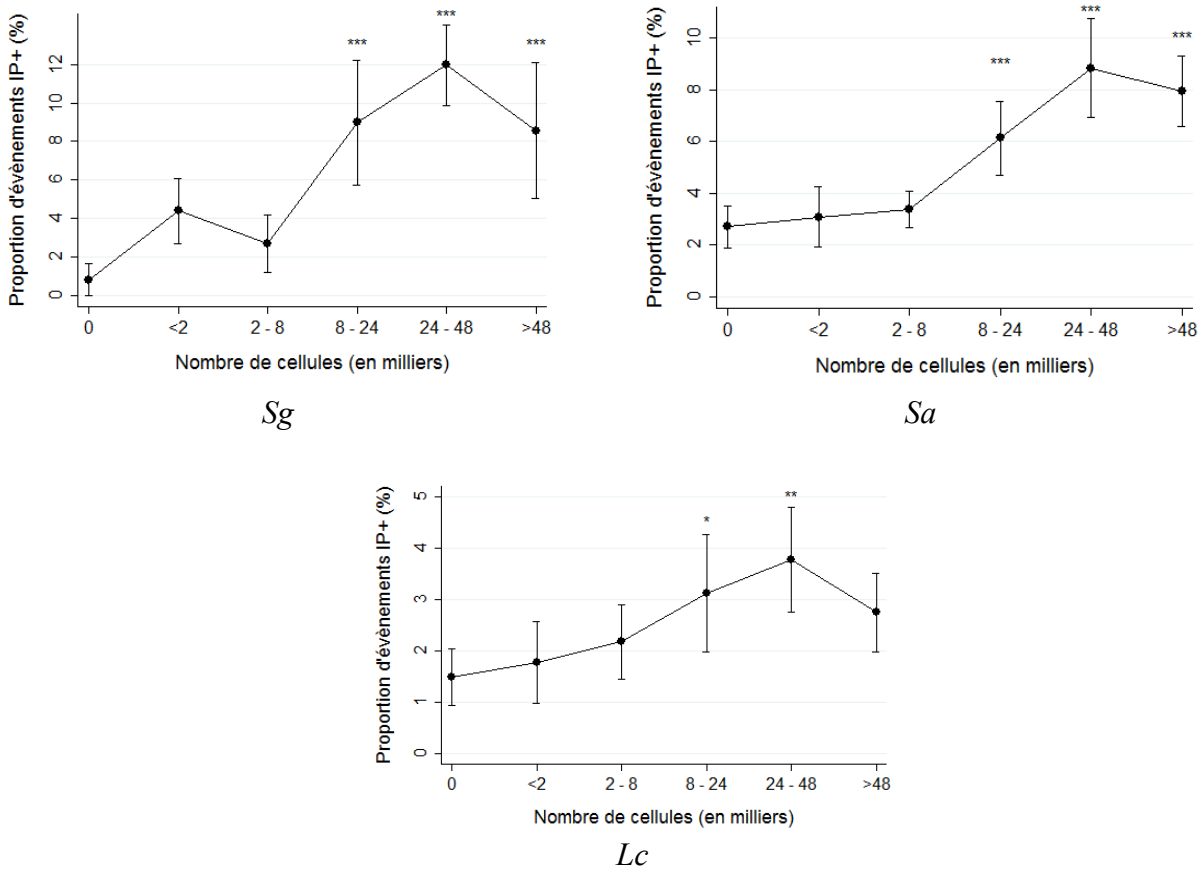


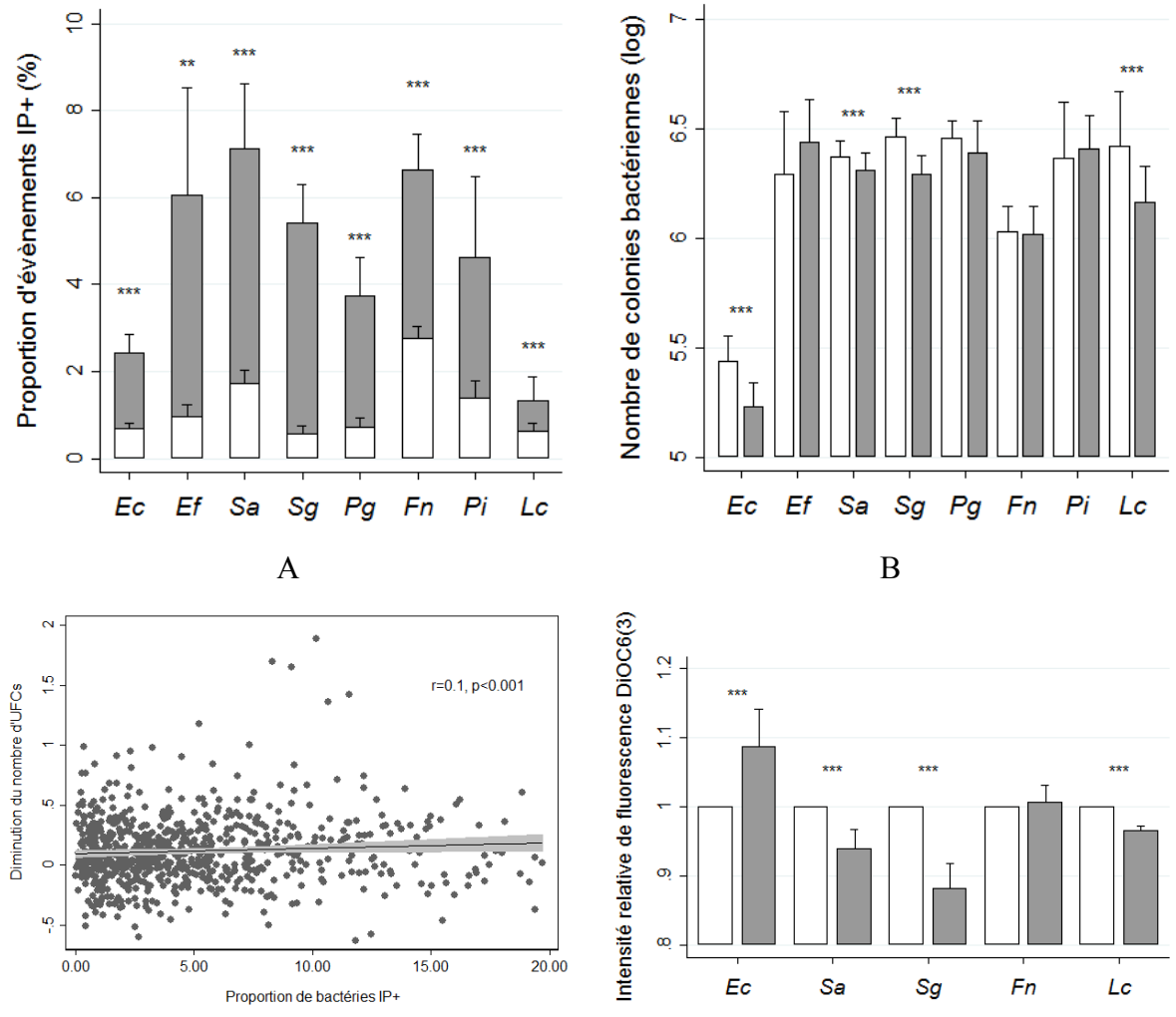
Figure II-7 : Les ASCs ont une activité antibactérienne dépendante du nombre de cellules. Différents nombres de cellules ont été infectés pendant 6 heures dans des plaques 12 puits avec la même quantité de bactéries. La proportion de bactéries IP+ a été mesurée par cytométrie en flux (N=9).

L'effet antibactérien est à spectre large et dépend de la charge bactérienne initiale

Pour toutes les souches, la proportion de bactéries IP positives était significativement augmentée après contact avec les ASCs (Figure II-8A, Figure II-9). Le nombre de d'UFCs pour *Ec*, *Sa*, *Sg* et *Lc* étaient également significativement diminué après 6 heures de contact avec les ASCs (Figure II-8B). La corrélation était significative entre la décroissance du nombre d'UFCs sur gélose et l'augmentation des évènements IP+ ($r=0.1$, $p<0.001$, Figure II-8C). Au contact des ASCs l'ensemble des souches de bactéries testées avaient la perméabilité de leur membrane augmentée et certaines limitaient leur prolifération.

La Figure II-10 montre que la dose initiale de bactéries influençait la capacité des ASCs d'induire une action antibactérienne, révélant un effet plus bactéricide que bactériostatique. La décroissance du ratio vert/rouge pour le marquage DiOC6(3) (permettant de s'affranchir du changement de taille des bactéries) pourrait indiquer une dépolarisation de la membrane des bactéries gram positives (Figure II-8D). Cette action antibactérienne a également été démontrée

par prélèvements sous-gingivaux humains ; les ASCs ont réduit de manière significative le nombre d'UFCs après que le milieu de culture a été infecté pendant 6 heures avec ces bactéries Figure II-11.



Equation de Gompertz modifiée

$$DO_{600} = A - exp\left\{-exp\left[\frac{\mu e}{A}(\lambda - t) + 1\right]\right\}$$

Souche	Groupe	N	Moyenne de µ	Sig
Ec	Sans ASCs	7	0.821 ± 0.134	*
	Avec ASCs		0.743 ± 0.140	
Sa	Sans ASCs	6	0.631 ± 0.254	*
	Avec ASCs		0.583 ± 0.233	
Sg	Sans ASCs	6	0.583 ± 0.233	*
	Avec ASCs		0.454 ± 0.170	

E

Figure II-8 : Les ASCs ont démontré un effet antibactérien à spectre large, et induisent une modification de la polarisation de la membrane, pour les souches gram positive et gram négatives. Différentes souches

bactériennes ont été mises en contact pendant 6 heures dans un milieu de culture α-MEM + 10% SVF sans (barres blanches) ou avec ASCs (barres noires) de manière aérobie ou anaérobie. **A –** Les ASCs ont inhibé la croissance des UFCs sur gélose. Après dilutions sériées, les bactéries ont été incubées sur agar pour comptage (N=10). **B –** Les ASCs ont été capable de modifier la perméabilité des membranes bactériennes. Les bactéries ont été marquées au Syto-62® et à l'iodure de propidium (IP), puis analysées par cytométrie en flux. Les changements de proportion de bactéries IP+ reflétaient les changements de la perméabilité de la membrane (N=10). **C –** Il existe une relation positive significative entre bactéries perméabilisées et diminution du nombre d'UFCs. **D –** Les ASCs induisent une modification de la polarisation des membranes bactériennes. Après marquage au DiOC6(3), le ratio Vert/Rouge a été utilisé pour analyser la modification du potentiel de membrane, indépendamment du changement de taille ou de forme des bactéries (N=9 à 15). **E –** Les ASCs ont modifié le taux de croissance des bactéries. Les paramètres ont été estimés en utilisant une régression par équation de Gompertz modifiée pour les trois souches *Ec*, *Sg* et *Sa*. La croissance bactérienne a été évaluée en monitorant la densité optique à 600 nm pendant 12 heures. Le temps est noté t, le taux de croissance μ, la densité optique maximale A, le temps de latence λ. Aucune différence significative n'a été observée pour les paramètres A et λ (N=6 à 7).

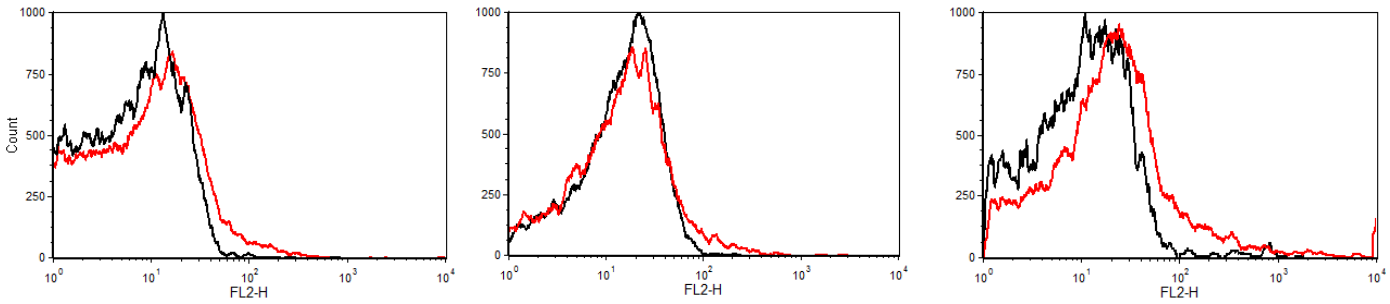
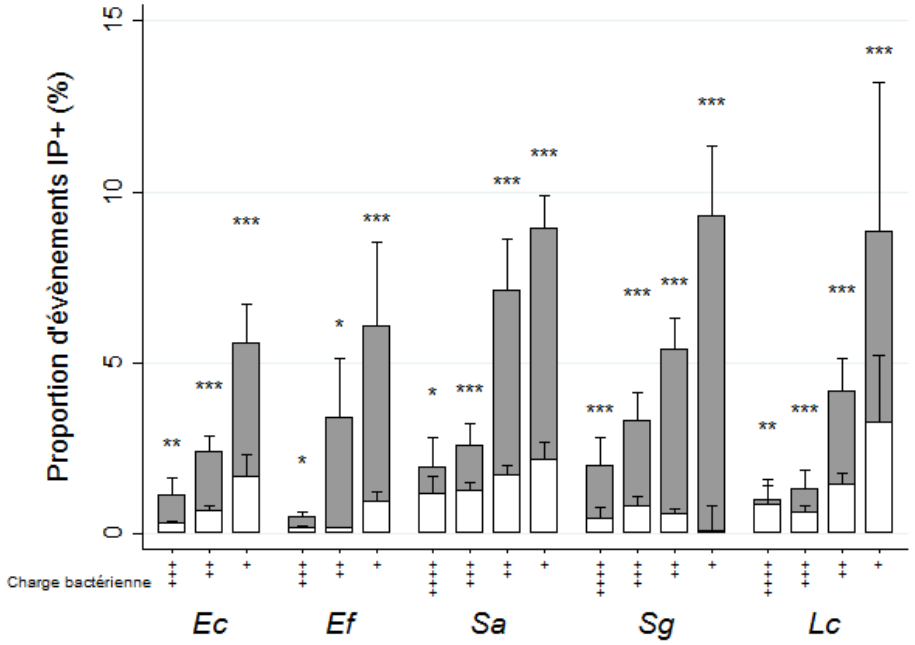
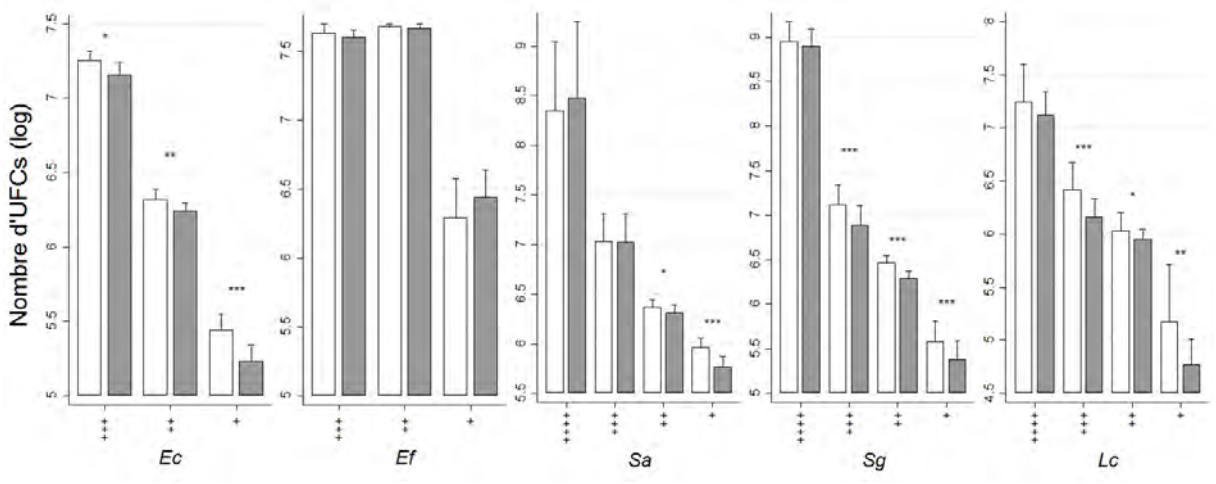


Figure II-9 : Les ASCs ont induit un changement au niveau de la fluorescence IP des bactéries. L'histogramme de fluorescence en FL2 est donné pour les bactéries seules (noir) et après contact avec les ASCs (rouge).



A



B

Figure II-10 : l'effet antibactérien à spectre large des ASCs était dépendant de la charge bactérienne initiale. Le milieu de culture a été infecté avec différentes doses de bactéries, sans (barres blanches) ou avec ASCs (barres grises) (N=6). **A –** La perméabilité membranaire bactérienne a été évaluée par cytométrie en flux comme précédemment décrit. **B –** Le nombre d'UFCs bactériennes a été évalué comme précédemment décrit (N=6).

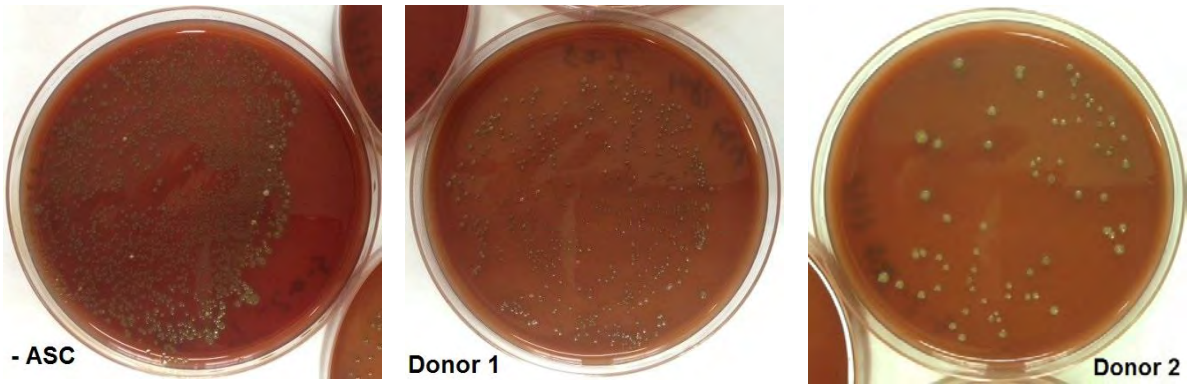


Figure II-11 : Infection d'ASCs avec des prélèvements sous-gingivaux de deux patients. Le contrôle est à gauche, exemple de deux donneurs d'ASCs infectés avec le même donneur de bactéries à droite.

L'effet de perméabilisation est en partie lié à la sécrétion de radicaux libres oxygénés

Etant donné l'effet de certains antioxydants sur la croissance bactérienne et donc sur la modification potentielle de l'effet antibactérien lié à cette modification de croissance, nous avons ajusté la proportion de bactéries IP+ sur le nombre de bactéries, en appariant sur le donneur de cellules.

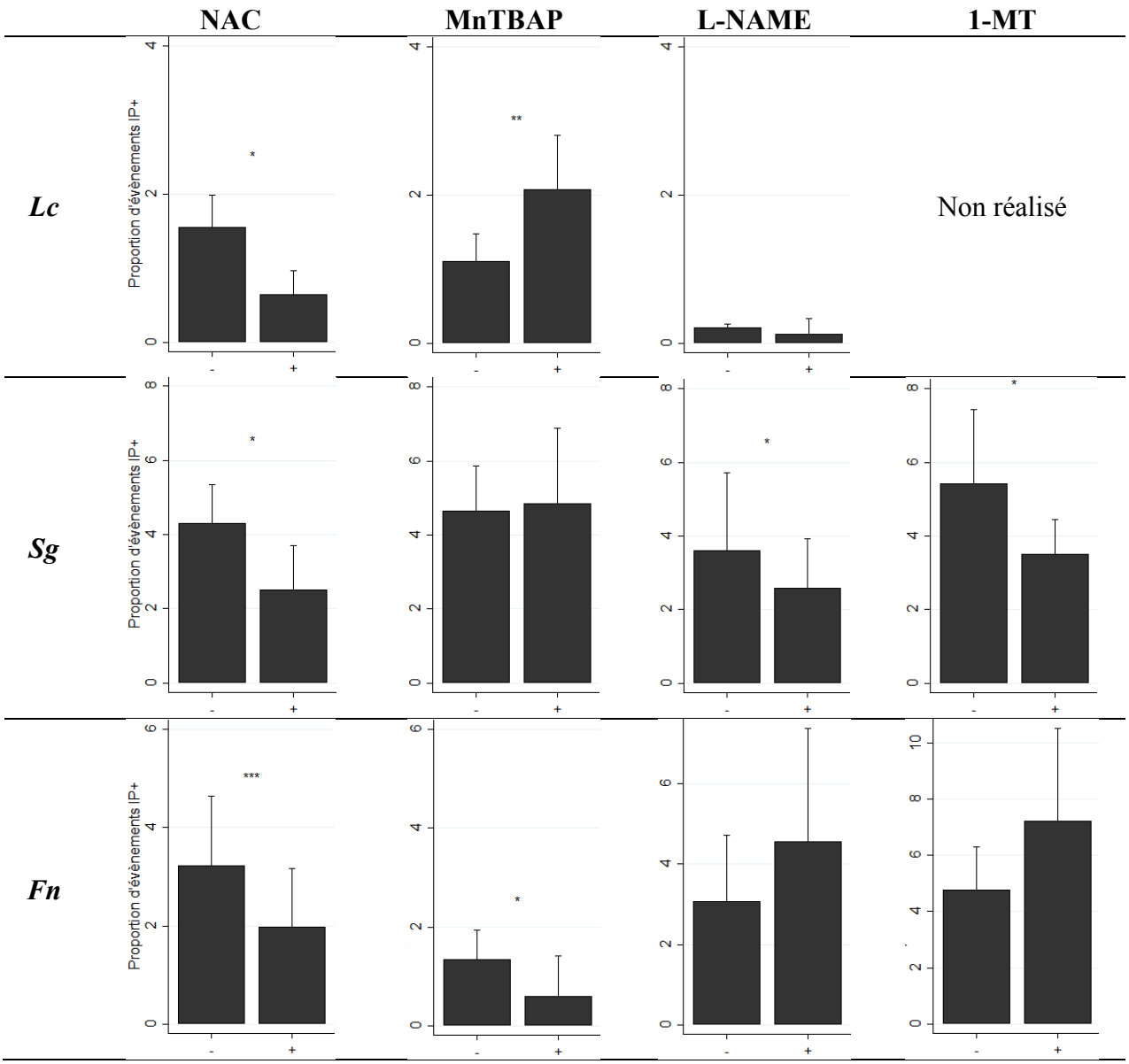


Figure II-12 : Antioxydants et effet antibactérien des ASC. La diminution significative de la mortalité bactérienne pour les 3 souches après 6 heures de contact semble indiquer qu'une partie de la perméabilisation de la membrane bactérienne passe par un effet des RLO. Pour chaque inhibiteur, la différence de proportion de bactéries IP+ est donnée sans et avec ASC (- et + respectivement) car l'utilisation de chaque inhibiteur nécessite un contrôle –ASC avec l'inhibiteur. NAC est la N-acétyl-cystéine, pan antioxydant ; MnTBAP est un mimétique de la SOD avec effet catalase-like et piégeur de peroxynitrite ; L-NAME est un inbiteur de la iNOS ; 1-MT ou 1-méthyl tryptophane est un inhibiteur de l'indoléamine 2,3-dioxygenase (IDO) (N=6 à 8).

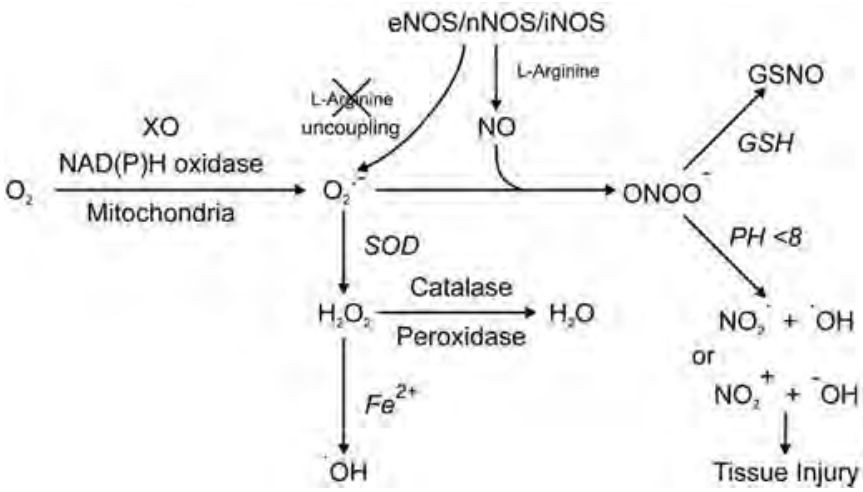
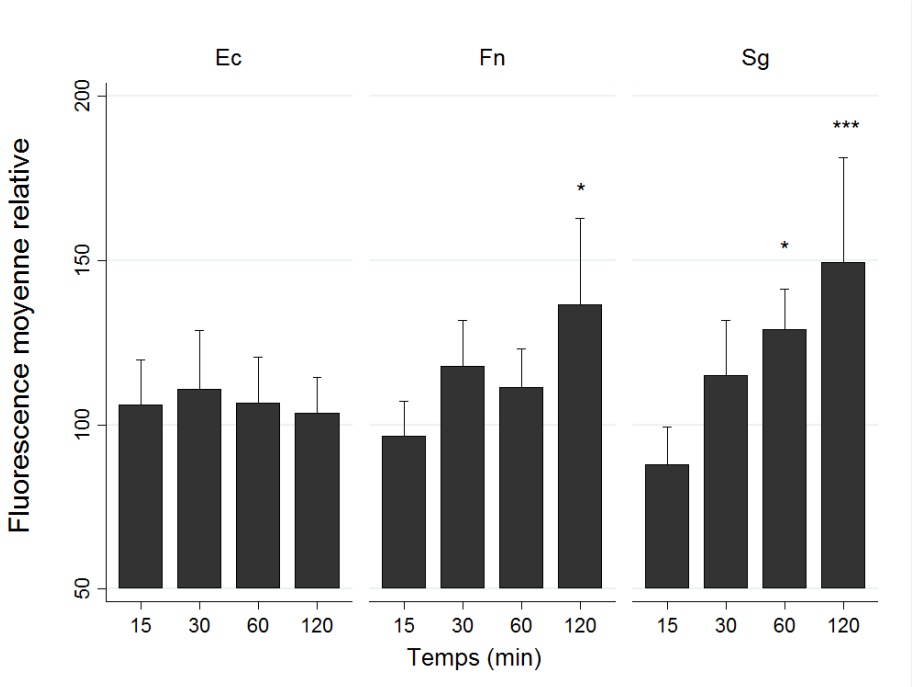


Figure II-13 : Schéma récapitulatif de la génération des principaux RLO.

La mise en contact des ASC avec trois souches bactériennes (*Lc*, *Sg* et *Fn*) avec ou sans certaines molécules antioxydantes, semble indiquer qu'une partie de la perméabilisation de la membrane bactérienne passe par un effet des RLO (Figure II-12, Figure II-13). En effet, il est observé une diminution significative de la proportion d'évènements IP+ lorsque la NAC est utilisée pour les 3 souches. Plus particulièrement le MnTBAP pour *Fn*, le L-NAME et 1-MT pour *Sg* diminuent également de manière significative ces proportions. Au contraire, l'utilisation de MnTBAP augmente la proportion d'évènements IP+ pour *Lc*.

A



B

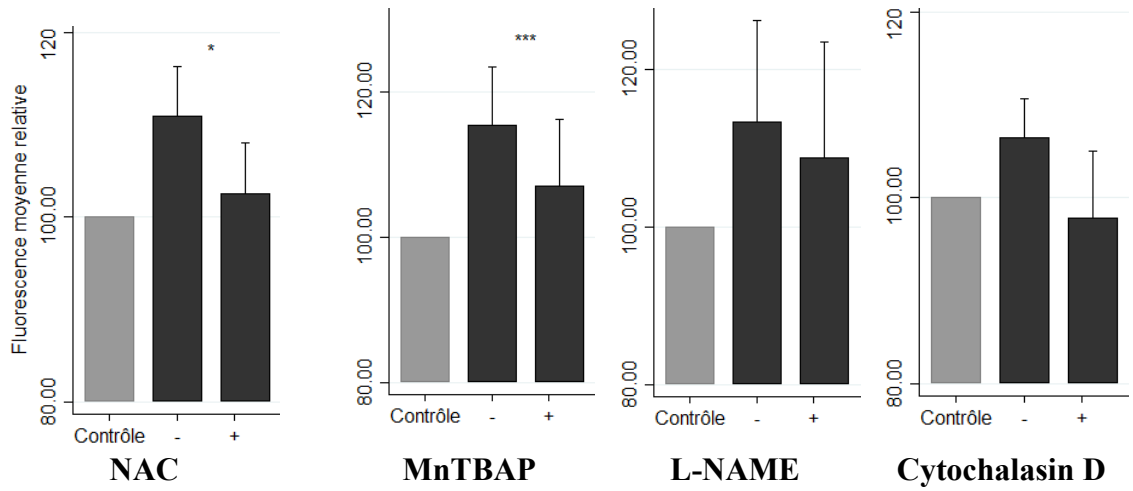


Figure II-14 : L'augmentation des RLO intra-cellulaires est dépendante du temps et de la souche bactérienne. **A-** Après marquage des ASC au H2DCFDA, les cellules ont été mises en contact avec *Ec*, *Fn* ou *Sg* pendant 15, 30, 60 ou 120 minutes. Les cellules ont été décollées et la fluorescence mesurée en cytométrie en flux (N=6). **B –** Avant et durant l'infection, le milieu de culture a été supplémenté en antixoydant (NAC, MnTBAP ou L-NAME) ou en cytochalasin D, et les cellules mises en contact ou non avec *Sg* pendant 60 minutes (N=6).

La Figure II-14 montre que le contact avec *Sg* induit une augmentation significative de la fluorescence au H2DCFDA des ASC à partir d'une heure. Cette fluorescence est diminuée si les antioxydants NAC et MnTBAP, ou la Cytochalasin D sont utilisés. L'augmentation de fluorescence n'est significative qu'à 2 heures pour *Fn* et non détectable pour *Ec*.

Les ASCs sont capables de perturber la division bactérienne

En prenant des microbilles en référence de FSC/SSC, nous avons observé des changements significatifs de taille et de granulosité des bactéries après contact avec les ASCs. Par exemple, FSC et SSC étaient augmentés pour *Sg*, *Fn* et diminués pour *Sa* (Figure II-16, Table II-4). Après 6 heures de contact avec les cellules, la cinétique de croissance a été suivie par densité optique, modélisée par une équation modifiée de Gompertz. Pour les trois souches étudiées (*Ec*, *Sg*, *Sa*), nous avons enregistré une diminution significative au niveau du paramètre taux de croissance (μ) après contact avec les ASCs alors que les autres paramètres n'étaient pas modifiés (Figure II-8E). Il ne semble pas exister de changement à la surface des bactéries, objectivables par MEB ou MET, comme des trous ou des cloques (Figure II-15). Par l'absence de passage de la β-galactosidase, il ne semble pas y avoir de rupture suffisante de la membrane d'*Ec* (Table II-5). Néanmoins, des anomalies de division de *Sa* ont été retrouvées après contact avec les ASCs,

avec la présence de multiples plans focaux de division, donnant lieu à des formations pseudo-multicellulaires. L'épaisseur de la paroi de *Sa* n'était pas significativement modifiée (Figure II-15).

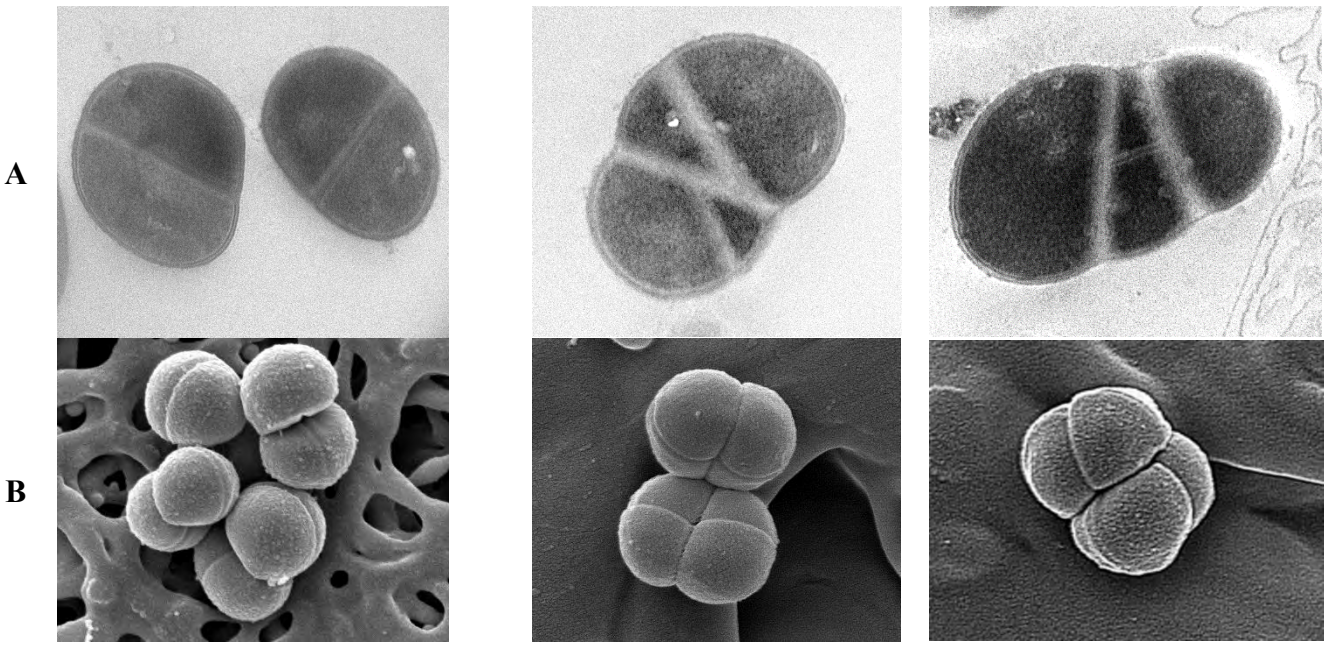


Figure II-15 : Les ASCs perturbent la croissance bactérienne avec une action au niveau membranaire. **A -** Images de microscopie électronique à transmission qui illustrent de multiples plans de division focaux lorsque les bactéries étaient mises en contact avec les ASCs. Aucune différence en terme d'épaisseur de la membrane n'a été mise en évidence (sans ASCs : 45.7nm±7.2 et avec ASCs : 51.5nm±13.6). **B –** L'imagerie électronique à balayage a confirmé des formations pseudo-multicellulaires lorsque *Sa* a été mis en contact avec les ASCs.

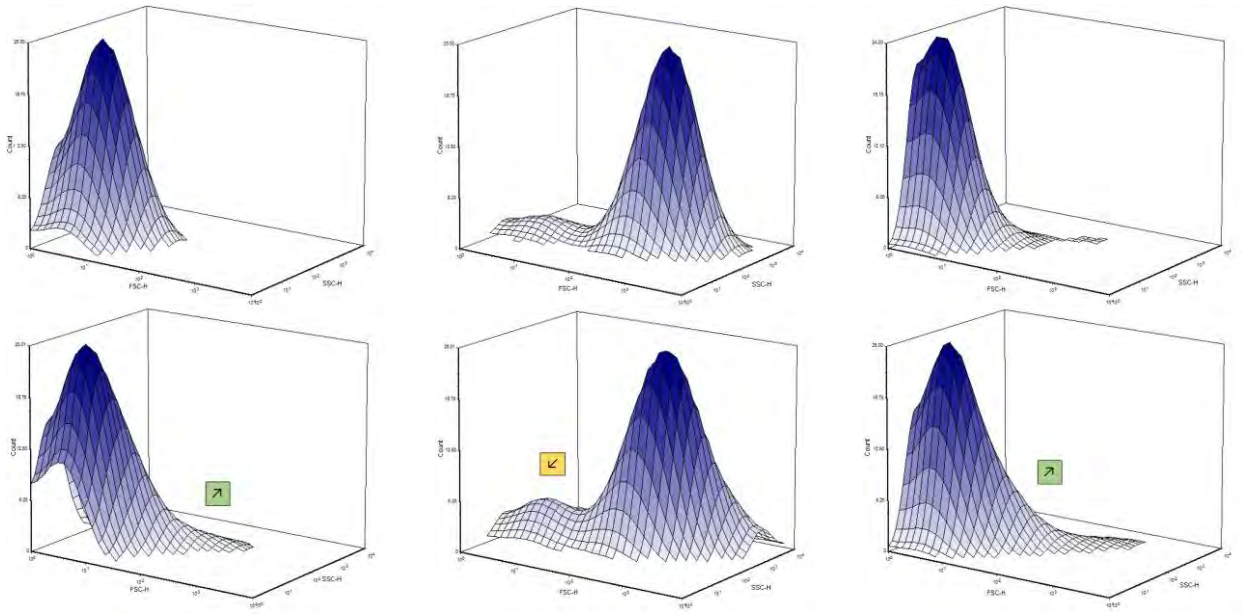


Figure II-16 : Les ASCs modifient la taille et la granulosité des bactéries. Ces histogrammes multidimensionnels montrent la variation de FSC et SSC pour trois souches bactériennes, sans contact (rangée du haut) ou avec contact (rangée du bas) avec les ASCs (N=10).

Souche	FSC sans ASCs	FSC avec ASCs	Sig.	SSC sans ASCs	SSC avec ASCs	Sig.
Ec	43.8 ± 6.55	44.2 ± 5.82	*ns*	40 ± 3.72	39.9 ± 5.57	*ns*
Ef	22.7 ± 6.95	27.8 ± 6.22	*	34.4 ± 7.65	35.9 ± 5.81	*ns*
Fn	27.4 ± 3.78	32.3 ± 3.96	***	39.3 ± 4.45	41.7 ± 6.68	***
Lc	40.5 ± 4.64	41.1 ± 3.88	*ns*	50.4 ± 1.39	49.4 ± 4.34	*ns*
Pg	27.2 ± 5.54	25.2 ± 4.26	*ns*	36 ± 4.79	34.1 ± 3.68	*
Pi	27.4 ± 3.93	28.5 ± 3.77	***	33.5 ± 2	33.5 ± 2.38	*ns*
Sa	84.5 ± 6.41	79.3 ± 5.32	***	64.8 ± 3.66	62.7 ± 3.85	***
Sg	25.4 ± 6.38	29.6 ± 6.16	***	35.2 ± 3.58	38.2 ± 6.91	***

Table II-4 : La table présente les valeurs obtenues de FSC et SSC pour chaque souche (avec ou sans ASCs). Ces valeurs ont été standardisées par rapport à des microbilles ajoutées, et considérées comme référence (N=10).

Souche	Groupe	N	Activité	Sig
Ec	Sans ASCs	8	6.51 ± 1.93	*
	Avec ASCs		3.32 ± 3.23	
Contrôle positif	Sans lyse d'Ec	8	6.01 ± 2.67	***
	Avec lyse d'Ec		22.32 ± 2.01	

Table II-5 : Les modifications membranaires provoquées par les ASCs ne permettent pas le passage de la β-galactosidase. Après une incubation des bactéries pendant 6 heures, avec ou sans contact avec les ASCs, le surnageant a été récupéré puis centrifugé deux fois. Après contact avec le substrat ONPG pour la β-galactosidase, la réaction a été monitorée par spectrophotométrie pour calculer l'activité enzymatique en variation de densité optique par unité de volume par minute. Pour générer le contrôle positif, une solution d'*Ec* a été lysée à l'aide de Tween 20 et de chloroforme.

Un contact direct est nécessaire pour induire une perméabilisation bactérienne

Après 6 heures d'incubation dans un milieu de culture avec ou sans ASCs, le milieu naïf (sans bactéries) ou conditionné (avec bactéries) a été récupéré. Ces milieux ont été infectés à nouveau pendant 6 heures, mais aucune différence de perméabilisation bactérienne n'a été démontrée (Figure II-17). Puisque cette absence d'effet pourrait être liée à la manière dont ont été traités les surnageants (centrifugation, puis filtration et congélation), un test de *transwell* 0.4µm a été mis au point afin de tester la contribution directe et indirecte des ASCs sur les bactéries (Figure II-18A). En comparant les parties externes du *transwell*, l'action directe des ASCs sur les trois souches testées a été confirmée. En comparant les parties internes du *transwell*, il existait une augmentation significative de la perméabilité des bactéries à l'IP pour *Fn* et *Sg* bien que cette proportion soit significativement plus faible que pour l'action directe (Figure II-18B).

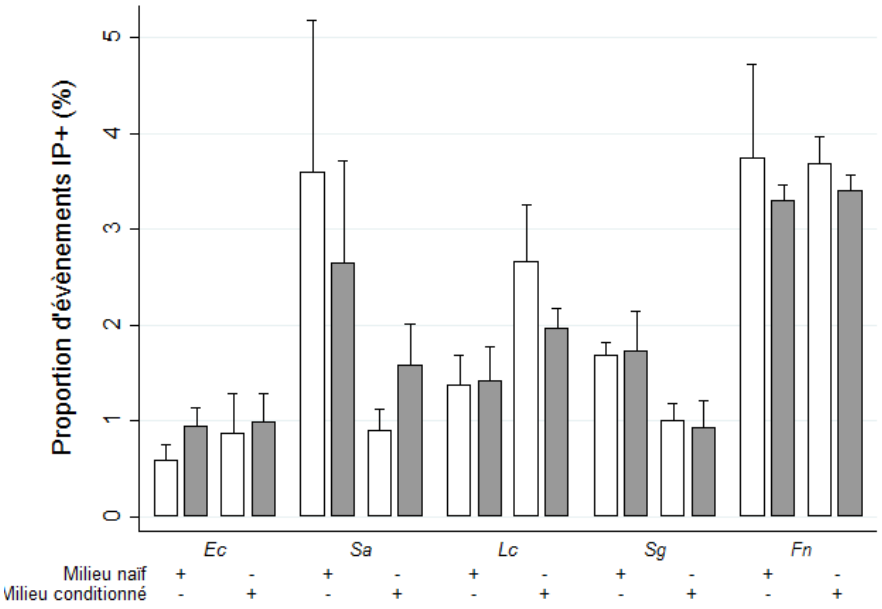
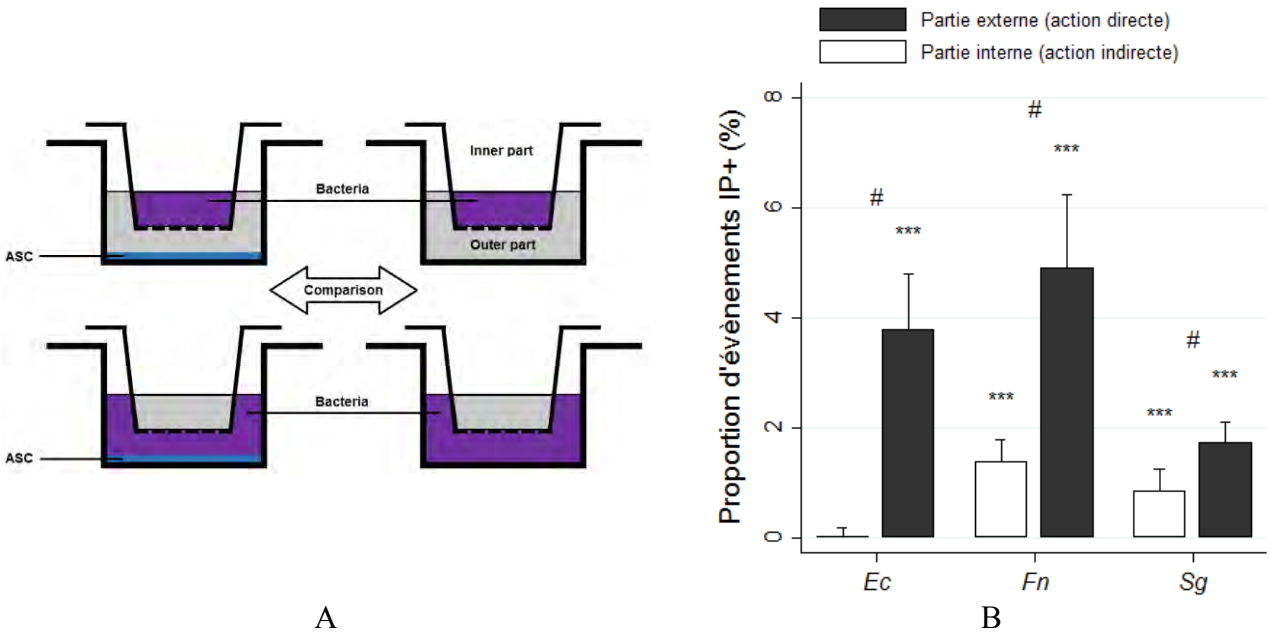


Figure II-17 : La présence des ASCs est nécessaire pour induire une perméabilisation membranaire. Après 6 heures d'incubation sans cellules (barres blanches) ou avec cellules (barres grises), du milieu naïf (sans bactéries) et du milieu conditionné (avec bactéries) a été collecté, filtré à 0.22µm et congelé à -20°C. Le milieu de culture a été réinfecté afin d'obtenir la concentration bactérienne requise, et incubé à nouveau pendant 6 heures (N=5).



A B

Figure II-18 : Un contact direct entre bactéries et ASCs est nécessaire pour obtenir l'effet antibactérien maximal. **A-** Résumé de l'expérimentation. L'observation au niveau de la partie externe a permis de mettre en évidence l'action directe des ASCs alors que la partie interne, l'action indirecte. **B –** Soit la partie interne (en blanc), soit la partie externe (en noir) d'inserts 12 puits 0.4µm ont été infectés avec 3 souches (*Ec*, *Fn* et *Sg*). Les codes * (* $p<0.05$, ** $p<0.01$, *** $p<0.001$) montrent une différence significative entre les conditions sans et avec ASCs. Le code # montre une différence significative entre la partie interne et externe (N=7).

Lorsque *Fn* est exposé à différentes doses d'ampicilline et de métronidazole durant 6 heures d'incubation, la présence d'ASCs diminue de manière significative le nombre d'UFCs en comparaison à l'absence d'ASCs (Figure II-19). *Fn* a été choisi car la diminution de la dose bactérienne n'a pas d'impact sur la mortalité induite par ASCs retrouvée sur gélose (données non montrées).

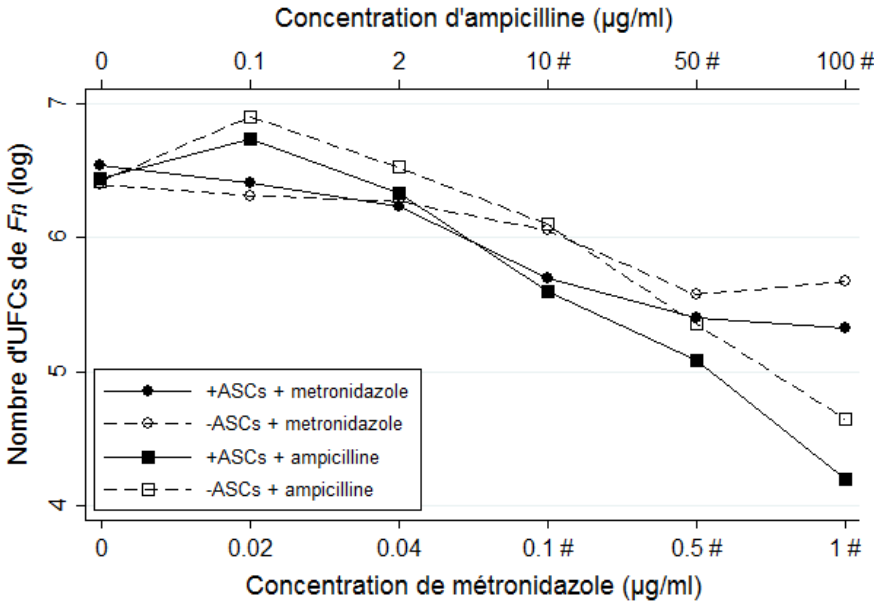


Figure II-19 : Les ASCs potentialisent l'action des antibiotiques. La souche *Fn* a été choisie car aucune inhibition sur gélose n'a été montrée, même à concentration bactérienne plus faible. Cette souche bactérienne a été mise en contact avec différentes concentrations de deux antibiotiques (ampicilline ou métronidazole), avec ou sans ASCs pendant 6 heures. Le code # montre une différence significative entre –ASCs et +ASCs pour un temps donné.

Analyse multivariée de la perméabilisation bactérienne

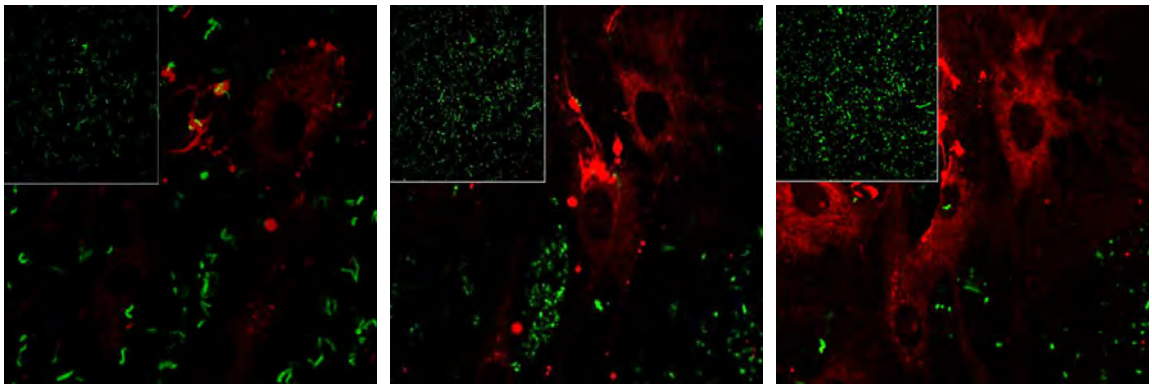
Pour *Fn*, indépendamment de l'âge, du nombre de bactéries et de son pourcentage d'évènements IP+ au niveau du contrôle, l'indice de masse corporelle était significativement associé au pourcentage d'évènements IP+ avec les ASC (coefficient de 2.18 avec un intervalle de confiance à 95% de [0.78 ; 3.57] et un nombre de 135 observations pour 37 patients). Pour *Sa*, indépendamment de l'indice de masse corporelle, du nombre de bactéries et de son pourcentage d'évènements IP+ au niveau du contrôle, l'âge était significativement associé au pourcentage d'évènements IP+ avec les ASC (coefficient de -0.17 avec un intervalle de confiance à 95% de [-0.31 ; -0.04] et un nombre de 212 observations pour 36 patients).

Les ASCs possèdent des capacités d'internalisation et de phagocytose

En utilisant un marqueur membranaire et des bactéries *Sg* marquées au CFSE, des acquisitions de microscopie *timelapse*, ont suggéré la capacité des ASCs à capturer et internaliser les bactéries. Nous avons observé une diminution des bactéries à partir d'environ 6 heures en comparaison au contrôle (Figure II-20A, Figure II-20B gauche). Dès 15 à 30 minutes,

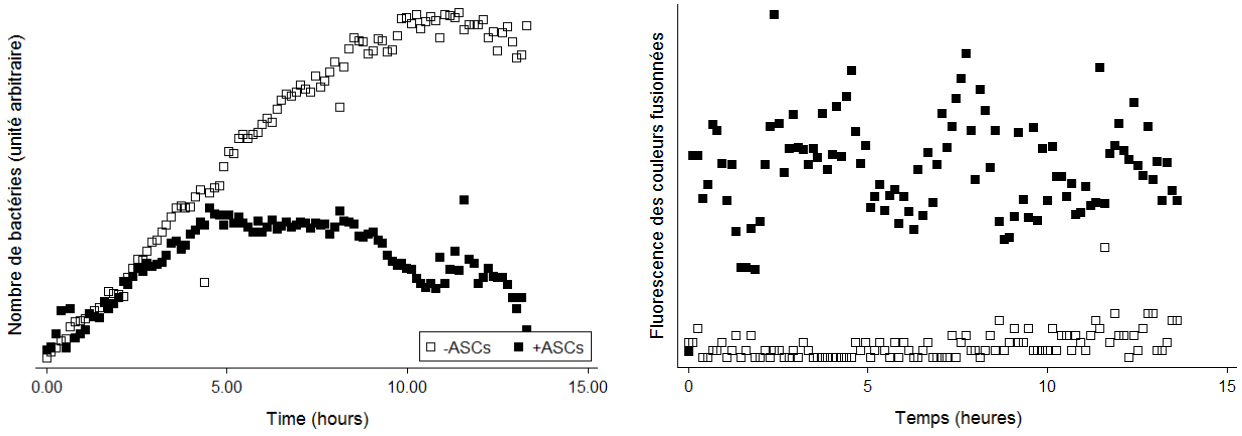
l'interaction cellules/bactéries était globalement constante (Figure II-20B droite). Des analyses en MEB suggéraient également l'internalisation de *Fn* par les bactéries (Figure II-20C) avec des zones préférentielles de fixation, notamment des prolongements cellulaires (Figure II-20D). Les bactéries ont été marquées avec un marqueur sensible au pH (l'intensité de fluorescence augmente de manière très importante avec la diminution du pH). Une augmentation significative du pourcentage de cellules positives en FL1 et de la fluorescence moyenne des cellules a été observée pour *Sg* et *Fn*. Cette augmentation a été abolie lorsque l'inhibiteur de la polymérisation de l'actine (cytochalasin D) a été utilisé (Figure II-20E). LAMP-1 (lysosome-associated membrane protein 1, CD107a) est une protéine N-glycosylée, exprimée de manière ubiquitaire dans les lysosomes et les endosomes tardifs. Cette protéine est impliquée dans la stabilité du lysosome et de son intégrité (128). La colocalisation intracellulaire entre le marquage LAMP-1 et les bactéries suggérait que *Fn* et *Sg* étaient contenus dans les phagolysosomes (Figure II-20F). Ceci est confirmé par les analyses en z-stack (Figure II-21).

A

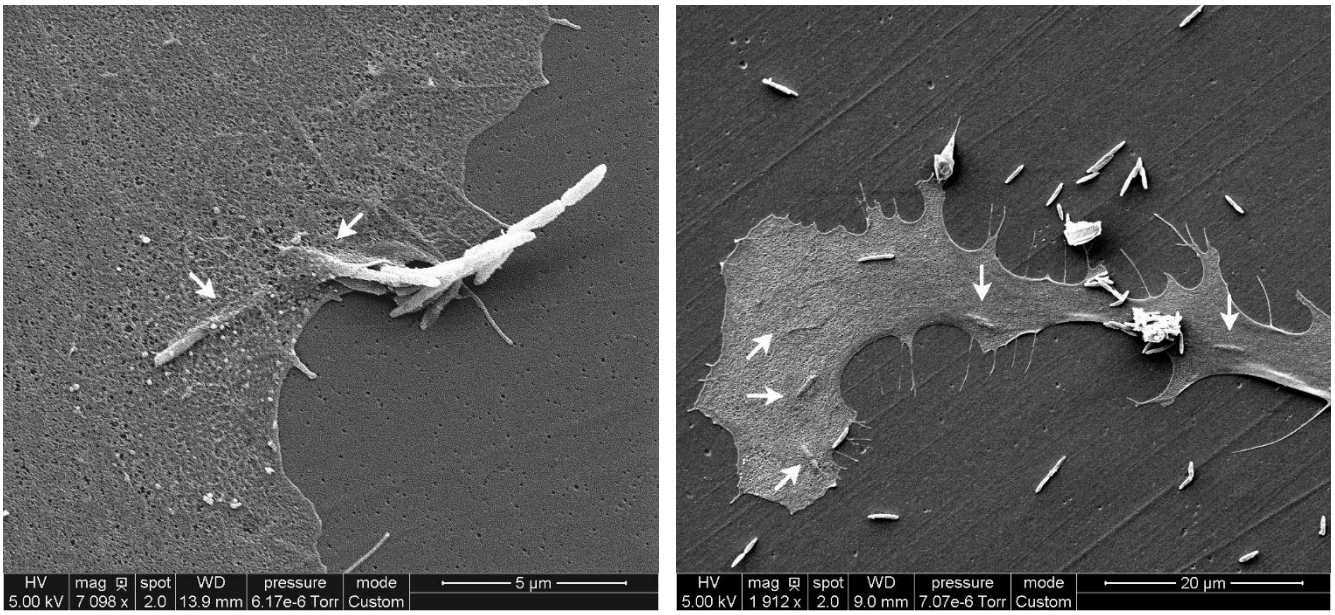


Baseline 6 heures 12 heures

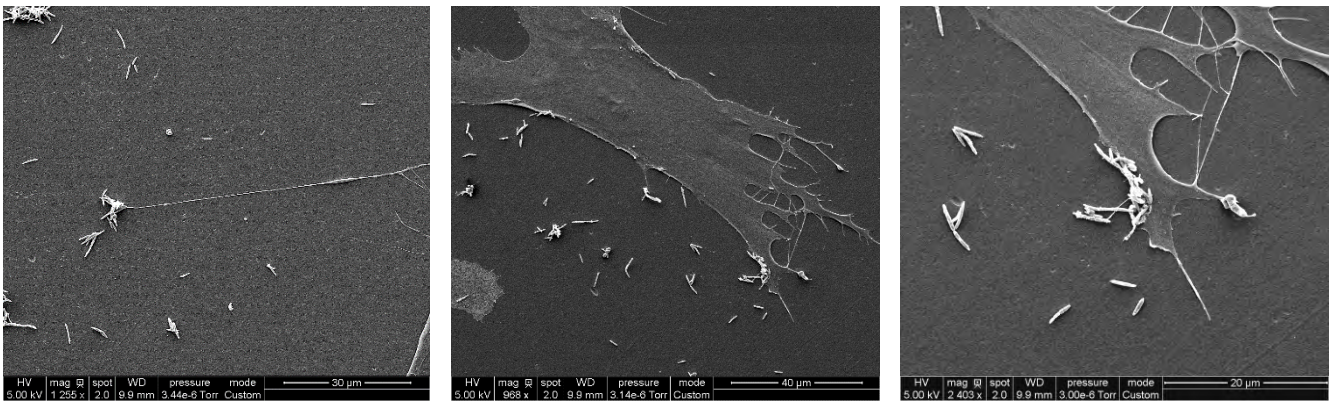
B



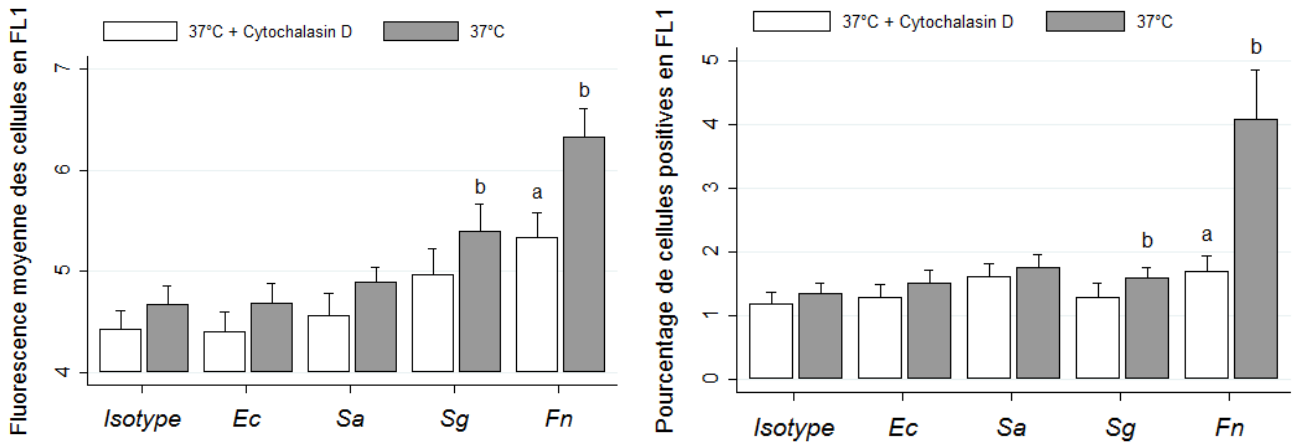
C


HV 5.00 kV | mag 7 098 x | spot 2.0 | WD 13.9 mm | pressure 6.17e-6 Torr | mode Custom | 5 µm
HV 5.00 kV | mag 1 912 x | spot 2.0 | WD 9.0 mm | pressure 7.07e-6 Torr | mode Custom | 20 µm

D


HV 5.00 kV | mag 1 255 x | spot 2.0 | WD 9.9 mm | pressure 3.44e-6 Torr | mode Custom | 30 µm
HV 5.00 kV | mag 968 x | spot 2.0 | WD 9.9 mm | pressure 3.14e-6 Torr | mode Custom | 40 µm
HV 5.00 kV | mag 2 403 x | spot 2.0 | WD 9.9 mm | pressure 3.00e-6 Torr | mode Custom | 20 µm

E


37°C + Cytochalasin D
37°C
Fluorescence moyenne des cellules en FL1
4
5
6
7
b
b
a
b
Isotype
Ec
Sa
Sg
Fn
37°C + Cytochalasin D
37°C
Pourcentage de cellules positives en FL1
0
1
2
3
4
5
b
b
a
b
Isotype
Ec
Sa
Sg
Fn

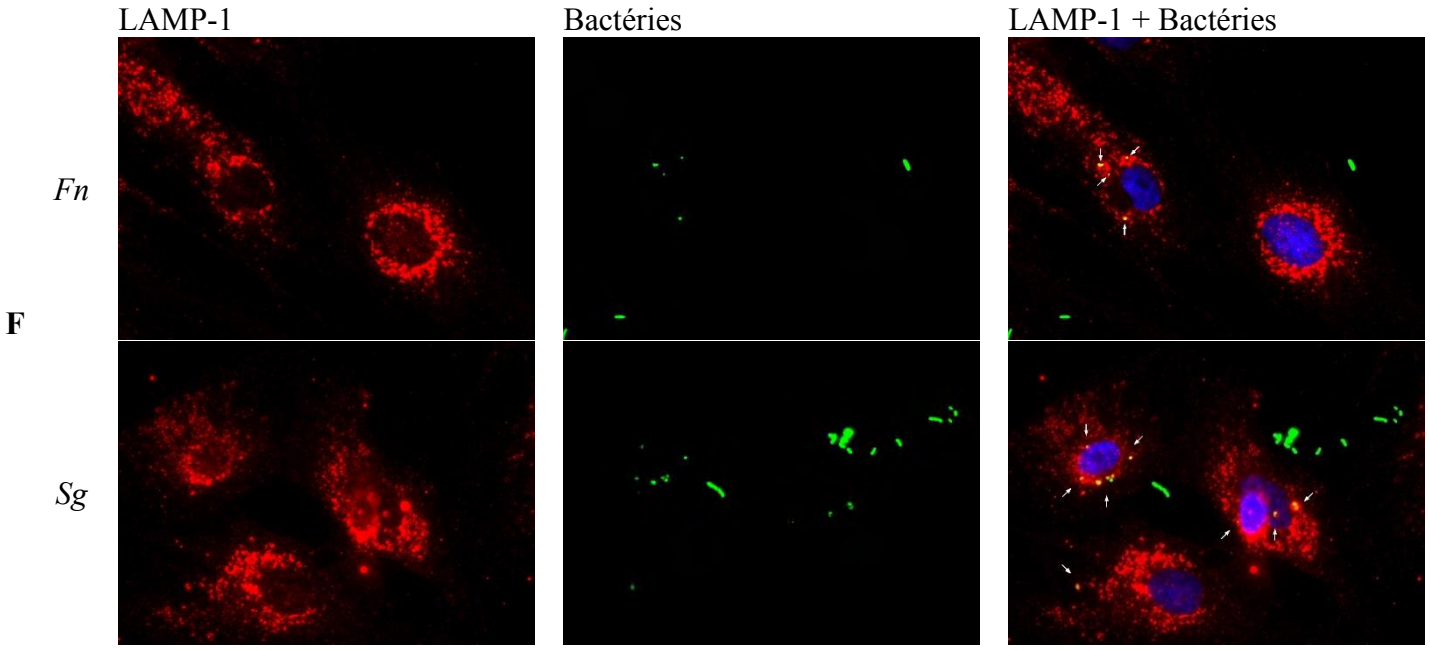


Figure II-20 : Les ASCs avaient des activités de type phagocytes-like. **A -** Captures de microscope en timelapse aux temps 0, 6 et 12 heures, avec ASCs (image principale) et sans ASCs (image incrustée dans le coin en haut à gauche). Les bactéries *Sg* ont été prélaablement marquées au CFSE (vert) et les cellules au CellTrace far red (rouge). Les intéractions entre bactéries et cellules apparaissent en jaune. N=4. **B -** Le nombre de particules vertes ont été extraites en utilisant ImageJ au fur et à mesure du temps (gauche), et le nombre de pixels jaunes ont été comptés (droite). **C -** Images de MEB montrant la pénétration de *Fn* dans les ASCs, ou *Fn* contenu dans les cellules (flèches blanches). **D -** *Fn* apparait en MEB comme intéagir avec des parties spécifiques de la membrane. **E –** Différentes souches bactéeriennes ou du PBS (isotype) ont été préléablement marquées avec le marqueur sensible au pH (pHRhodo). Les cellules ont été infectées à une multiplicité d'infection de 1 pour 100 durant 1 heure, avec ou sans l'inhibiteur du cytosquelette, la cytochalasin D à 0.4µM. En utilisant la cytométrie en flux, la fluorescence moyenne et le pourcentage de cellules positives en FL1 on été respectivement représentées à gauche et à droite. Le code « a » indique une différence significative entre les conditions expérimentales avec la cytochalasin D et le contrôle isotype respectif, et le code « b », une différence significative entre les conditions expérimentales sans la cytochalasin D et le contrôle isotype respectif (N=7). **F –** Les ASCs ont été infectés avec des bactéries marquées au CFSE (vert) à 1 :100 pendant 1 heure. Les cellules ont été fixées et marquées au LAMP-1 (rouge). La colocalisation apparait en jaune (flèches blanches à droite) (N=4).

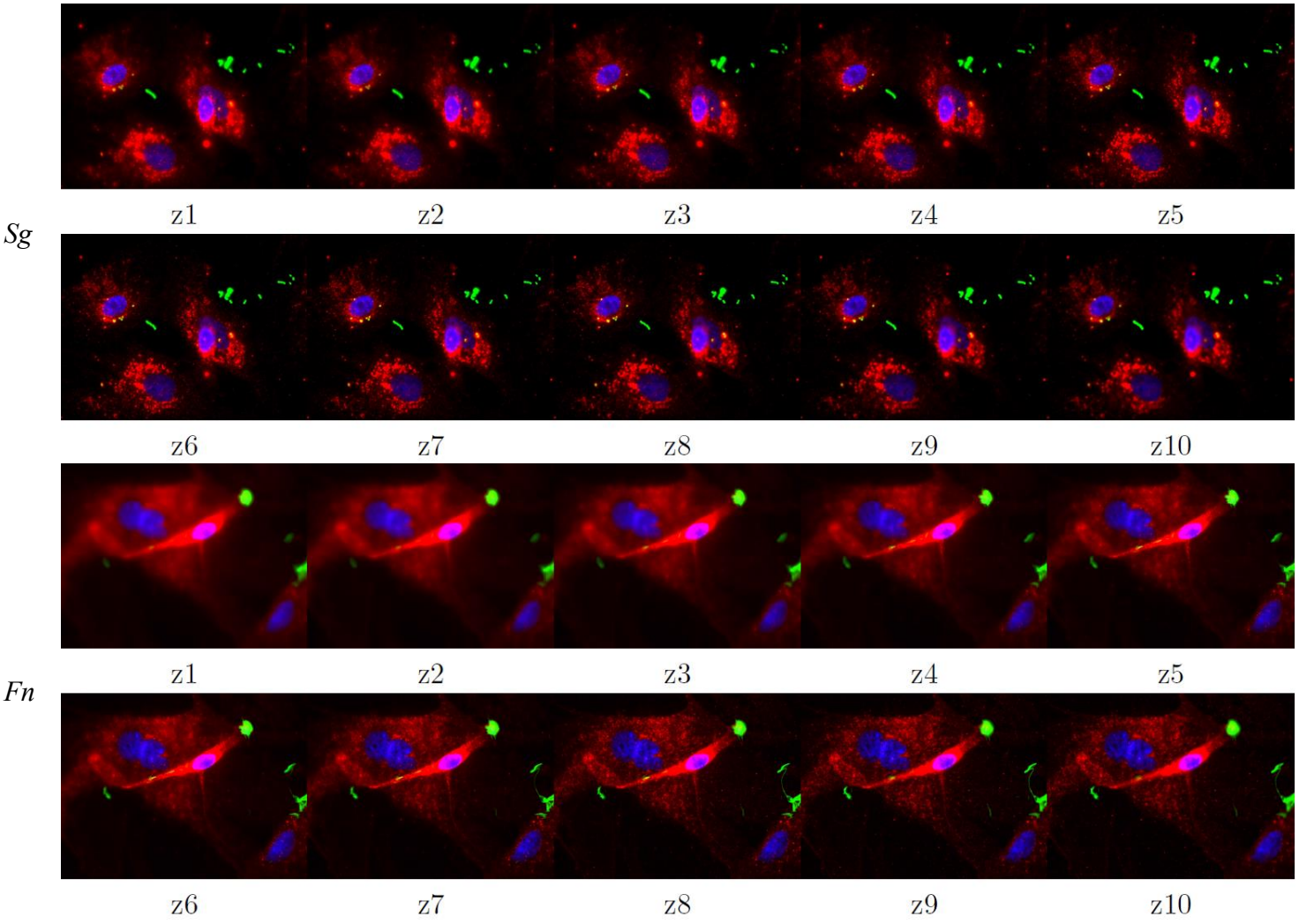


Figure II-21 : *Sg* et *Fn* étaient contenus dans les phagolysosomes, à l'intérieur des cellules. Acquisitions en z-stack.

Le contact des ASCs avec les bactéries induit l'expression des médiateurs de l'inflammation, des TLRs et de peptides antibactériens (Table II-6)

Nous avons étudié si le contact des ASCs avec des bactéries gram négatives et positives modifiait l'expression de mécanismes de défenses antibactériens potentiels (peptides antibactériens comme les β-défensines, le dérivé cathélicidine LL-37, ou encore le BPI *bactericidal/permeability-increasing protein*), de certains PAMPs (TLR 2-3-4), chimiokines, cytokines, récepteurs impliqués dans un effet pro et anti-inflammatoire.

L'analyse en qPCR révèle une augmentation de l'expression de certains gènes impliqués dans la réponse pro-inflammatoire (IL1β, IL-6, IL33, TNFα) et anti-inflammatoire (IL-10), et ceci avec plusieurs souches. La réponse chimiotactique est également augmentée (MCP-1 et

CXCL2). Uniquement testé pour *Fn*, il existe une augmentation importante (et significative) de l'expression d'IFNβ (activité impliquée dans la réponse antivirale, antiprolifération et immuno-modulatrice). De plus, il existe une augmentation significative du TLR3 et 4 au niveau de *Fn*, et de TLR2 et 4 au niveau de *Sg*. Concernant les peptides antibactériens, nous n'avons pas mis en évidence d'augmentation de l'expression de β-défensines. L'expression de la cathélicidine LL-37 était significativement augmentée pour Sa et *Sg*, mais pas pour *Fn*.

Fn				
Gene	**N**	**Fold change moyen ± SD**	**Variation**	**P**
TLR2	7	1.74 ± 1.22		0.18
TLR3	7	1.84 ± 0.84	+	0.02
TLR4	7	1.24 ± 0.32	+	0.05
HBD1	7	1.18 ± 1.08		0.74
HBD3	7	1.29 ± 0.91		0.40
LL37	7	3.05 ± 5.63		0.35
MCP1	7	16.1 ± 6.34	+	0.02
IL1β	7	144.3 ± 134.4	+	0.02
IFNβ	7	87.0 ± 115.6	+	0.02
Sa				
TLR2	8	1.15 ± 0.57		0.48
TLR3	7	0.83 ± 0.28		0.13
TLR4	7	0.91 ± 0.24		0.32
HBD3	7	0.98 ± 0.44		0.50
LL37	7	2.72 ± 1.94	+	0.04
MCP1	7	1.79 ± 1.21	+	0.02
CXCL2	7	10.7 ± 5.60	+	0.02
IL1β	8	9.04 ± 11.3	+	0.02
IL6	7	2.21 ± 1.42	+	0.05
IL33	7	1.78 ± 0.73	+	0.02
TNFα	7	4.06 ± 3.47	+	0.02
IL10	7	4.49 ± 1.84	+	0.02
Sg				
TLR2	7	2.51 ± 1.30	+	0.04
TLR3	7	0.77 ± 0.31		0.18
TLR4	7	1.19 ± 0.22	+	0.05
HBD3	7	0.88 ± 0.53		0.40
LL37	7	2.56 ± 2.56	+	0.05
MCP1	7	8.19 ± 13.2		0.18
CXCL2	7	8.81 ± 12.1	+	0.02
IL1β	7	64.9 ± 123.0	+	0.02
IL6	7	4.51 ± 5.49	+	0.02
IL33	7	1.92 ± 0.89	+	0.02
TNFα	7	22.3 ± 32.3	+	0.02
IL10	7	11.4 ± 10.5	+	0.02

Table II-6 : expression de certains gènes en RT-PCR. Certains gènes n'ont pas encore été testés pour les 3 souches.

Effet antibactérien des ASCs dans un modèle murin de parodontite

Comme détaillé en II.2.3, nous avons utilisé le modèle de parodontite par gavage bactérien oral pour déterminer si la charge bactérienne était modifiée suivant que le coté ait été greffé avec les ASCs ou non.

Le nombre d'UFCs (en échelle log) était significativement diminué (Figure II-22) du côté greffé par les ASCs par rapport au côté contrôle (3.53 ± 0.37 versus 3.86 ± 0.24, p=0.002). Deux espèces bactériennes principales, gram positive, catalase négative, ont pu être identifiées comme étant potentiellement des *Streptococcus xylosus* et *Streptococcus sciuri* (galerie apiStaph, Biomérieux).

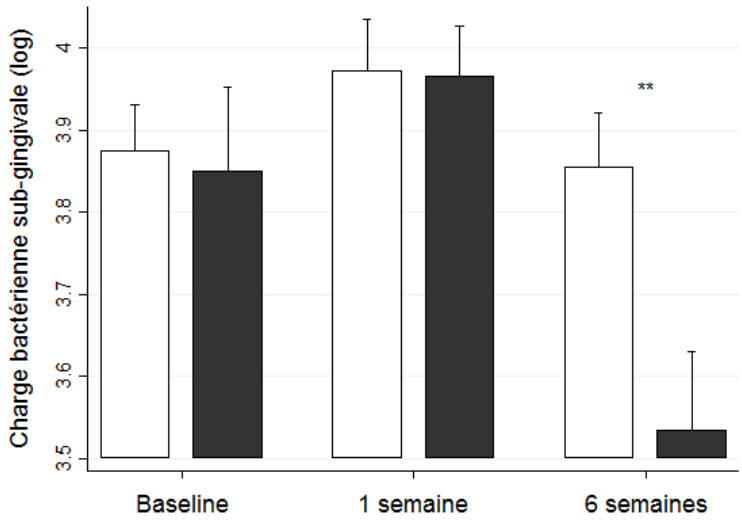


Figure II-22 : quantification de la flore sous-gingivale de la souris (exprimée en log d'UFC). On oberve une diminution significative à 6 semaines.

II.3.5 Discussion et perspectives

Les données suggèrent des effets antibactériens des ASC à large spectre liés à de multiples mécanismes. Cet effet est positivement corrélé au nombre de cellules, négativement corrélé au nombre de bactéries initiales et maximal à 6 heures dans les conditions de culture utilisées pour ces expérimentations.

Un rôle des RLO complexe à étudier

Les ASC sont capables de générer des radicaux libres oxygénés, comme les macrophages, par l'intermédiaire d'un mécanisme NADPH dépendant (129-131). La production de radicaux libres oxygénés semble être impliquée dans la perméabilisation bactérienne bien que de nombreux facteurs rendent cette analyse difficile. Certaines souches bactériennes comme *Streptococcus sanguinis* produisent de l'H_2O_2 et sont donc capables d'augmenter la fluorescence du H2DCFDA situé en intracellulaire, comme le montre la diminution de la fluorescence après traitement à la cytochalasin D. Leur production pourrait être liée à l'internalisation bactérienne puisque la souche non-enteroinvasive *Ec* - ATCC25922 ne montre pas d'augmentation du signal H2DCFDA ou pHrodo, en comparaison à *Fn* et *Sg*.

La production de RLO est également dépendante du mode de respiration. L'augmentation de l'expression de la superoxide dismutase dans des conditions aérobies pourrait influer sur les espèces radicalaires produites par les cellules et par les bactéries (132). Une manière d'explorer ce phénomène serait d'utiliser la même souche bactérienne, et de l'exposer à des conditions aérobie ou anaérobie. Dans une expérimentation préliminaire, nous avons ainsi observé une diminution de la mortalité bactérienne en anaérobiose par rapport à l'aérobiose pour *Sg*, suggérant un rôle de l'anion superoxide. De plus, une analyse multivariée sur les 3 parodonto-pathogènes anaérobies suggère un rôle protecteur de la peroxydase bactérienne, à la fois sur la perméabilisation bactérienne et le nombre d'UFCs. La production d'H_2O_2 pourrait donc être un des mécanismes de toxicité sur ces souches en anaérobiose. Comme nous le suggérons au niveau de *Sg*, la génération de NO par la iNOS stimulée par IDO pourrait expliquer une partie de l'effet des ASC sur cette souche. Ce mécanisme de toxicité par NO/iNOS et TLR2 dépendant sur *Streptococcus spp.* a été montré par exemple avec des cellules odontoblastiques (133).

Les RLO peuvent aussi être des seconds messagers du signal intracellulaire, et interférer avec les processus cellulaires, comme la prolifération, la migration, différenciation et sécrétions paracrines (134, 135). Leur génération pourrait également exercer des effets nocifs sur les cellules elles-mêmes, à l'origine d'une modulation de l'expression/sécrétions de molécules antibactériennes. La génération de RLO augmente par exemple le potentiel régénératif des ASC, particulièrement sur l'angiogenèse à travers la production de VEGF (136, 137) et induit la différenciation des ASC en adipocytes en favorisant l'accumulation lipidique (138).

Deux autres difficultés de la compréhension de l'effet des RLO viennent du fait que les bactéries soient vivantes (seul moyen de corréler la modulation de production de RLO par des

antioxydants et la perméabilisation bactérienne), et que le blocage d'une partie des RLO pourrait favoriser une autre voie de leur production (par exemple le blocage de la iNOS inhibe la production de dérivés du monoxyde d'azote, et donc des peroxynitrites, favorisant la production d'H_2O_2).

Les ASC sont capables de phagocytose mais cet évènement n'est pas majoritaire

Kriebel *et al.* ont rapporté dans un modèle de culture anaérobie que *Fn* était capable d'envahir des BM-MSC et de stimuler la sécrétion de l'IL-8 (139). La présence de *Fn* en intracellulaire n'est pas spécifique des CSM ; en effet, cette espèce bactérienne est capable d'envahir d'autres types cellulaires comme les cellules gingivales (140). Les propriétés d'ingestion et de microbicidie de *Candida parapsilosis* ont été montrées sur des preadipocytes 3T3-L1 en utilisant de l'acridine orange et du cristal violet comme marqueur de viabilité (141). Preadipocytes et CSM partagent également de nombreux gènes en commun avec les macrophages (122). Dans cette étude, des bactéries gram positives et négatives ont été retrouvées dans des phagolysosomes. Pourtant, ces évènements sont relativement rares. Une quantification automatisée par Operetta permettra de confirmer les résultats observés avec le pHrodo. Si la phagocytose a un faible impact sur la mortalité, l'internalisation bactérienne est très certainement un évènement déclencheur. En effet plusieurs données sous-tendent cette théorie. Les expérimentations en *transwell* ont montré la nécessité d'un contact cellules-bactéries pour obtenir le maximum de perméabilisation bactérienne. La microscopie à balayage suggère également des sites de fixation préférentiels pour *Fn*. Les ASC expriment des récepteurs de motifs moléculaires associés aux pathogènes (PAMP) comme la famille des TLR (*toll-like receptors*), impliqués dans la détection des composants bactériens et l'activation des cellules immunitaires (142). Les ASC expriment ainsi TLR-1 à TLR-6 et TLR-9 (143). Les lipopolysaccharides de *Ec* et les peptidoglycanes de *Sa* augmentent la différenciation ostéogénique des ASC (144). Les conditions de cultures hypoxiques augmentent l'expression du TLR-1, 2, 5 et 9. Le TLR-1 reconnait un champ large de pathogènes, le TLR-2 les composants des bactéries gram-positives, le TLR-5 les flagellines bactériennes et le TLR-9 le motif CpG de l'ADN bactérien (144). Les effets bénéfiques des ASC en thérapie cellulaire pourraient donc être modulés selon la pression partielle en oxygène et le microbiome de l'environnement.

Les expressions des TLR3 et 4 sont augmentés après contact avec *Fn*. Ceci peut être rapproché de résultats obtenus avec des monocytes (145) avec une augmentation de l'expression du TLR3 par le LPS (agoniste du TLR4) via une voie TLR4-MyD88-IRAK-TRAF6-NF-κB-dependante (145, 146). Au contraire, l'expression du TLR3 est down-régulée après stimulation par du sBLP (145)(lipopeptide bactérien synthétique agoniste du TLR2 (147)). Nous obtenons une tendance à la diminution pour nos souches gram positives.

Bien que l'expression de l'IFNβ n'ait été regardée encore que pour *Fn*, nous observons son augmentation, comme attendu (Figure II-23).

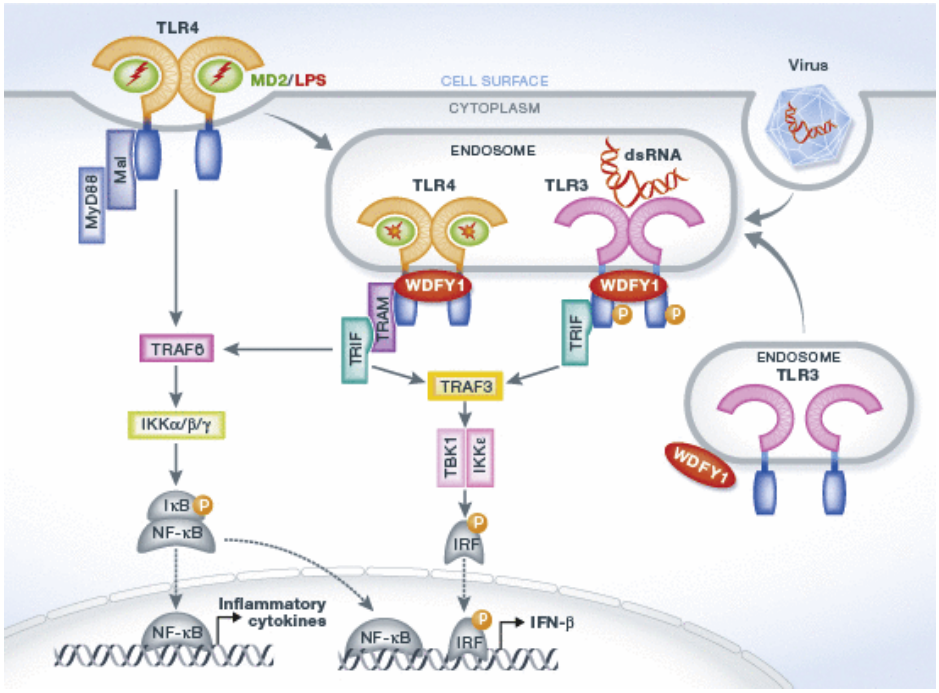


Figure II-23 : Les voies TLR4 (MyD88 dépendante) et TLR3 (MyD88 indépendante) induisent la production d'IFNβ sous le contrôle de NF-κB (148).

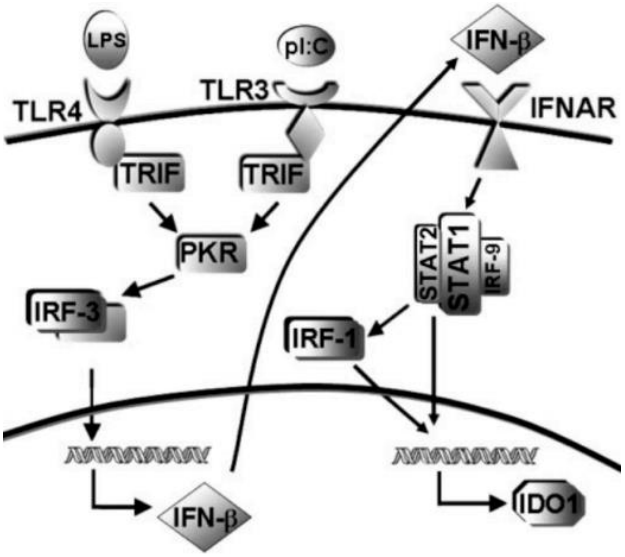


Figure II-24 : IFNβ permet d'augmenter l'expression de l'indoleamine 2,3-dioxygenase (IDO). IDO possède à la fois une action immunomodulatrice et antibactérienne (via la NO synthase inductible)(149).

L'induction d'IFNβ serait à l'origine d'une augmentation de l'expression de l'indoléamine 2,3-dioxygenase (IDO) (Figure II-24). IDO possède à la fois une action immunomodulatrice et antibactérienne (via la NO synthase inductible)(149). La diminution de la perméabilité bactérienne après utilisation du piégeur de peroxynitrite MnTBAP (avec une activité SOD mimétique et catalase) ou après utilisation de L-NAME ou de 1-MT, pourrait aller en ce sens.

De nombreux paramètres restent donc à explorer : il sera donc nécessaire de comprendre le rôle de TLR2 et 4 dans la fixation et l'internalisation bactérienne, le rôle de TLR2/4 dans la stimulation de l'IFNβ, de l'IDO et de la iNOS. L'utilisation d'anticorps bloquants dirigés contre ces TLR et l'IFNβ, l'utilisation de l'inhibiteur de l'iNOS (le L-NAME) en testant différentes concentrations, permettra de mieux comprendre les voies d'activation mises en jeu.

L'activité antibactérienne des ASC est liée à un contact entre bactéries et cellules et à la sécrétion paracrine de facteurs antibactériens

Comme déjà détaillé, l'analyse en *transwell* suggère que le contact entre bactéries et ASC est nécessaire. Une perspective de ce travail consisterait à mettre en place des bactéries à la fois dans la partie interne et externe du *transwell*, avec ou sans ASC. Ceci permettrait de déterminer sur les bactéries de la partie interne, si l'effet pourrait être purement lié la présence d'un facteur paracrine antibactérien déclenché par le contact ou si le contact en soi serait responsable de l'activité antibactérienne.

Un article récent a identifié le peptide antimicrobien de la famille des cathélicidines, le LL-37, comme responsable de l'activité anti-*Sa* d'adipocytes (150, 151). Néanmoins, les préadipocytes n'ont pas montré cette capacité. Dans cette étude nous avons identifié une augmentation significative de l'expression de LL-37 après contact avec *Sa* ou *Sg*, mais pas *Fn*. Pourtant les observations cliniques sont en faveur de l'importance de ce peptide dans la protection des tissus parodontaux et gingivaux contre les pathogènes (152). Ainsi, la déficience congénitale de LL-37 dans le syndrome de Kostmann (153) ou l'absence de la forme mature de LL-37 dans le syndrome de Papillon-Lefèvre (154) semble être la raison majeure pour le développement précoce de parodontites sévères chez ces patients. *In vitro*, *Fn* montre la plus grande sensibilité à ce peptide par rapport aux autres souches gram négatives et positives testées (155). Il sera nécessaire de réaliser un western blot de cette protéine pour confirmer ces résultats. D'autres auteurs ont montré des capacités *in vitro* des BM-MSCs à inhiber la croissance d'*Escherichia coli* par l'intermédiaire du LL-37 (156). L'exploration de ce peptide et des voies de signalisation impliquées sera donc nécessaire.

Suite à l'article de Zhang et al. (151), Miller résume les principales voies de recherche qui pourraient être suivies afin d'analyser l'effet antibactérien des adipocytes (157). Ces pistes de recherche sont également transposables aux ASCs :

- Quels mécanismes conduisent à la production de LL-37 ?
- Le signal est-il direct, via des récepteurs PAMP (comme les TLR), ou indirect par le biais d'un signal pro inflammatoire d'autres cellules ?
- De quelle manière le peptide précurseur subit-il une maturation ? Existe-il une différence entre celui produit par les ASC et celui produit par les adipocytes ?
- La vitamine D a été montrée comme importante pour induire la cathélicidine et promouvoir la réponse immune chez l'hôte. La vitamine D a-t-elle donc un rôle dans la production de LL-37 par les ASCs ? La 1,25-dihydroxyvitamin D3 (1,25(OH)2D3), le métabolite actif vitamine D, régule l'expression de nombreux gènes mis en jeu dans la défense de l'hôte et les fonctions immunes (158). L'enzyme qui métabolise la 1,25(OH)2D3 à partir de la 25(OH)D3 est la 1α-hydroxylase (1α-OHase : CYP27B1). Or chez les monocytes/macrophages, 1α-OHase est exprimée en réponse à l'activation par IFNγ ou par le TLR, et induit l'expression du VDR (récepteur de la vitamine D, nucléaire) et la sécrétion de 1,25(OH)2D3. La vitamine D active ainsi la dimérisation de VDR avec RXR (récepteur rétinoïde X), stimulant de fait l'expression du LL-37 (158). L'activation de 1α-OHase augmente également l'expression de l'iNOS et de RANTES (159). Au contraire,

l'hypoxie diminue l'expression d'iNOS, et de CYP1A (cytochrome P450 dont le CYP27B1 en est une isoforme)(160). Ainsi, l'absence d'augmentation de l'expression de LL-37 pourrait être liée à ces éléments dans un environnement hypoxique. L'utilisation de l'inhibiteur du cytochrome P450, le kétoconazole, avec ou sans supplémentation du milieu en 1,25(OH)2D3, permettrait d'apporter des éléments en faveur de cette hypothèse (161). De manière intéressante, le prétraitement de BM-MSC avec de la 1,25(OH)2D3 permettrait de diminuer l'expression de cytokines pro-inflammatoires après mise en contact des cellules avec *Sa* résistant à la méthicilline (162). Il nous faudra néanmoins avant tout tester son expression au niveau des ASC.

Les modifications de la perméabilité bactérienne induisent des changements au niveau de la physiologie des bactéries. La dépolarisation des grams positifs mime ainsi les phénomènes observés lors du contact avec certains antibiotiques (163). Même si cela peut paraitre surprenant, l'hyperpolarisation observée pour *Ec* est également marqueur d'une perte de viabilité bactérienne ayant été démontrée par exemple lors de stress alcalins, de capture de protons et d'hyperconsommation d'ATP (164).

Comme l'augmentation de la perméabilité bactérienne est une stratégie pour favoriser l'action antibiotique sur des souches résistantes, nous avons souhaité déterminer si les ASC pouvaient favoriser l'action antibiotique sur *Fn*, une souche qui n'était pas impactée au niveau viabilité sur gélose. Le fait que les ASC sensibilisent *Fn* à l'action de l'ampicilline et du métronidazole ajoute un argument en faveur de molécules sécrétées capables de se lier à la double couche lipidique externe et perturber son organisation (165). Cette action pourrait s'avérer d'autant plus utile que la régénération tissulaire pourrait avoir lieu dans un environnement où les bactéries résiduelles seraient résistantes ou peu sensibles aux antibiotiques (par exemple *Staphylococcus spp.* résistants à la méthicilline au niveau d'ulcérations cutanées chez les patients diabétiques (166)).

De nombreux peptides cationiques, comme les défensines, ont été montrés comme potentiellement impliqués dans cette action, bien que les buffers utilisés pour les expérimentations aient une faible force ionique (167). La « *bactericidal/permeability-increasing protein »* ou BPI par contre (situé dans les granules des polynucléaires) est capable de se lier aux LPS avec un K_D de l'ordre du micro molaire (167). La modification de la force

ionique du milieu de culture apporterait des éléments quant à la nature des molécules antibactériennes impliquées (167).

L'augmentation de la perméabilité bactérienne n'entraine pas forcement la perte de la viabilité de la bactérie (168), paradigme différent de celui observé au niveau des cellules eucaryotes. La perméabilité révélée par l'IP est donc un marqueur sensible des dommages aux cellules, mais il n'est pas un indicateur de la mort cellulaire des bactéries stressées (même si, comme nous le montrons, il existe une corrélation)(168). Cet effet semble plutôt bactéricide que bactériostatique comme le sous-tend l'absence de modification du paramètre λ lors de la phase de remise en culture de bactéries exposées aux ASC (169).

II.3.6 Conclusion

Les données concernant l'effet antibactérien résultant directement d'une action des CSMs sont peu retrouvées dans la littérature, et à part dans notre équipe, non documentées pour les ASCs.

Les effets des CSMs pourraient donc être liés à une phagocytose des cellules (141, 170), à une production du peptide antibactérien LL-37 (151, 156), de la β-défensine 2/voie TLR-4 (171), Lipocalin-2 (172) et une participation du système iNOS et IDO (et une déplétion du tryptophane dans le milieu)(173). Néanmoins peu de micro-organismes ont été testés (*Candida spp, Escherichia coli*, *Pseudomonas aeruginosa* et *Staphylococcus aureus*). Ce travail démontre une action antibactérienne sur un plus large panel de bactéries (4 gram négatives et 4 gram-positives). Cet effet est dépendant du nombre de cellules, par phagocytose, ou sécrétion de radicaux libres oxygénés et de molécules antibactériennes (sécrétion nécessitant un contact préliminaire avec les bactéries).

Bien qu'un effet antibactérien des CSMs *per se* puisse être à l'origine de la diminution des colonies bactériennes observées dans le modèle *in vivo* de parodontite, l'interaction avec les acteurs du système de l'immunité est sans doute le mécanisme à explorer. Les CSM sont en effet capables de protéger de la septicémie à travers la stimulation de l'activité des monocytes circulants, protégeant ainsi du choc septique chez la souris (156, 174, 175). Une co-culture ASC/bactéries/macrophages, permettrait de mieux comprendre les effets potentiels *in vivo*.

Dans un article récent, Zhang et al. (151) identifient une sous-population de BMSC IL17+, ayant une capacité candidicide supérieure aux IL17-. Lorsqu'on bloque l'IL17, l'effet anti-*Candida* est significativement diminué. Il est donc très probable qu'une partie de l'action des

CSMs passe par l'activation de la voie NFκB, via une stimulation auto-paracrine IL-17 (151) ou IFNγ (173). Il existe également une augmentation de l'activité anti-*Candida* de la fraction vasculaire stromale de patients obèses comparée à des patients non-obèses (176). Ces 3 articles nous amènent donc à penser qu'il existe une grande hétérogénéité des sous-populations cellulaires à avoir une action antimicrobienne, et que le fait d'avoir un profil pro-inflammatoire, favorise cet effet. Il existerait donc au sein des tissus, un équilibre se créant entre les populations de CSMs tournées vers l'immunomodulation/immunosuppression et vers l'immunostimulation/antimicrobien.

Au final, les mécanismes antimicrobiens sont multiples, très certainement dépendants du type cellulaire, de l'environnement cellulaire initial (obésité, inflammation chronique, populations cellulaires en présence dont macrophages), fonction de l'agent infectieux (gram positif ou négatif, biochimie de l'agent), de la pression partielle en oxygène du milieu et de l'environnement cellulaire de greffe (quantités d'inflammation et d'infection restantes).

II.4 Bibliographie

1. Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. Lancet. 2005;366(9499):1809-20.

2. Savage A, Eaton KA, Moles DR, Needleman I. A systematic review of definitions of periodontitis and methods that have been used to identify this disease. J Clin Periodontol. 2009;36(6):458-67.

3. Leroy R, Eaton KA, Savage A. Methodological issues in epidemiological studies of periodontitis - how can it be improved? BMC Oral Health. 2010;10:8.

4. Bourgeois D, Llodra J, Nordblad A, Pitts N. Health surveillance in Europe - a selection of essential oral health indicators. Technical report, European Global Oral Health Development Project (EGOHID), 2005.

5. Albandar J. Periodontal disease surveillance. J Periodontol. 2007;78(7):1179-81.

6. Eke PI, Dye BA, Wei L, Thornton-Evans GO, Genco RJ, CDC Periodontal Disease Surveillance workgroup: James Beck (University of North Carolina CH, U. S. A.), Gordon Douglass (Past President, American Academy of Periodontology), R.y Page (University of Washin. Prevalence of periodontitis in adults in the United States: 2009 and 2010. J Dent Res. 2012;91(10):914-20.

7. Seymour GJ, Gemmell E, Reinhardt RA, Eastcott J, Taubman MA. Immunopathogenesis of chronic inflammatory periodontal disease: cellular and molecular mechanisms. J Periodontal Res. 1993;28(6 Pt 2):478-86.

8. Kolenbrander PE, Palmer RJ, Jr., Periasamy S, Jakubovics NS. Oral multispecies biofilm development and the key role of cell-cell distance. Nat Rev Microbiol. 2010;8(7):471-80.

9. Hajishengallis G, Liang S, Payne MA, Hashim A, Jotwani R, Eskan MA, et al. Low-abundance biofilm species orchestrates inflammatory periodontal disease through the commensal microbiota and complement. Cell Host Microbe. 2011;10(5):497-506.

10. Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: from the natural environment to infectious diseases. Nat Rev Microbiol. 2004;2(2):95-108.

11. Flemming HC, Wingender J. The biofilm matrix. Nat Rev Microbiol. 2010;8(9):623-33.

12. Svensäter G, Bergenholtz G. Biofilms in endodontic infections. Endodontic Topics. 2004;9(1):27-36.

13. Beikler T, Flemmig TF. Oral biofilm-associated diseases: trends and implications for quality of life, systemic health and expenditures. Periodontol 2000. 2011;55(1):87-103.

14. Christersson LA, Zambon JJ, Genco RJ. Dental bacterial plaques. Nature and role in periodontal disease. J Clin Periodontol. 1991;18(6):441-6.

15. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL, Jr. Microbial complexes in subgingival plaque. J Clin Periodontol. 1998;25(2):134-44.

16. Colombo AP, Boches SK, Cotton SL, Goodson JM, Kent R, Haffajee AD, et al. Comparisons of subgingival microbial profiles of refractory periodontitis, severe periodontitis, and periodontal health using the human oral microbe identification microarray. J Periodontol. 2009;80(9):1421-32.

17. Camelo-Castillo A, Novoa L, Balsa-Castro C, Blanco J, Mira A, Tomas I. Relationship between periodontitis-associated subgingival microbiota and clinical inflammation by 16S pyrosequencing. J Clin Periodontol. 2015.

18. Abdellatif HM, Burt BA. An epidemiological investigation into the relative importance of age and oral hygiene status as determinants of periodontitis. J Dent Res. 1987;66(1):13-8.

19. Loe H, Anerud A, Boysen H, Morrison E. Natural history of periodontal disease in man. Rapid, moderate and no loss of attachment in Sri Lankan laborers 14 to 46 years of age. J Clin Periodontol. 1986;13(5):431-45.

20. Teles R, Teles F, Frias-Lopez J, Paster B, Haffajee A. Lessons learned and unlearned in periodontal microbiology. Periodontol 2000. 2013;62(1):95-162.

21. Serino M, Blasco-Baque V, Nicolas S, Burcelin R. Managing the manager: gut microbes, stem cells and metabolism. Diabetes Metab. 2014;40(3):186-90.

22. Garlet GP, Cardoso CR, Campanelli AP, Ferreira BR, Avila-Campos MJ, Cunha FQ, et al. The dual role of p55 tumour necrosis factor-alpha receptor in Actinobacillus actinomycetemcomitans-induced experimental periodontitis: host protection and tissue destruction. Clin Exp Immunol. 2007;147(1):128-38.

23. Garlet GP. Destructive and protective roles of cytokines in periodontitis: a re-appraisal from host defense and tissue destruction viewpoints. J Dent Res. 2010;89(12):1349-63.

24. Lee JY, Sojar HT, Sharma A, Bedi GS, Genco RJ. Active domains of fimbrillin involved in adherence of Porphyromonas gingivalis. J Periodontal Res. 1993;28(6 Pt 2):470-2.

25. Wright HJ, Matthews JB, Chapple IL, Ling-Mountford N, Cooper PR. Periodontitis associates with a type 1 IFN signature in peripheral blood neutrophils. J Immunol. 2008;181(8):5775-84.

26. Tomomatsu N, Aoki K, Alles N, Soysa NS, Hussain A, Nakachi H, et al. LPS-induced inhibition of osteogenesis is TNF-alpha dependent in a murine tooth extraction model. J Bone Miner Res. 2009;24(10):1770-81.

27. Maekawa T, Krauss JL, Abe T, Jotwani R, Triantafilou M, Triantafilou K, et al. Porphyromonas gingivalis manipulates complement and TLR signaling to uncouple bacterial clearance from inflammation and promote dysbiosis. Cell Host Microbe. 2014;15(6):768-78.

28. Potempa M, Potempa J, Kantyka T, Nguyen KA, Wawrzonek K, Manandhar SP, et al. Interpain A, a cysteine proteinase from Prevotella intermedia, inhibits complement by degrading complement factor C3. PLoS Pathog. 2009;5(2):e1000316.

29. Taxman DJ, Swanson KV, Broglie PM, Wen H, Holley-Guthrie E, Huang MT, et al. Porphyromonas gingivalis mediates inflammasome repression in polymicrobial cultures

through a novel mechanism involving reduced endocytosis. J Biol Chem. 2012;287(39):32791-9.

30. Mayo C. Focal infection of dental origin. Dental Cosmos. 1922;64:1206-8.

31. Kumar PS. Oral microbiota and systemic disease. Anaerobe. 2013;24:90-3.

32. Newman HN. Focal infection. J Dent Res. 1996;75(12):1912-9.

33. Paquette DW, Madianos P, Offenbacher S, Beck JD, Williams RC. The concept of "risk" and the emerging discipline of periodontal medicine. J Contemp Dent Pract. 1999;1(1):1-8.

34. Nabet C, Lelong N, Colombier ML, Sixou M, Musset AM, Goffinet F, et al. Maternal periodontitis and the causes of preterm birth: the case-control Epipap study. J Clin Periodontol. 2010;37(1):37-45.

35. Offenbacher S, Beck JD, Jared HL, Mauriello SM, Mendoza LC, Couper DJ, et al. Effects of periodontal therapy on rate of preterm delivery: a randomized controlled trial. Obstet Gynecol. 2009;114(3):551-9.

36. Simpson TC, Needleman I, Wild SH, Moles DR, Mills EJ. Treatment of periodontal disease for glycaemic control in people with diabetes. Cochrane Database Syst Rev. 2010(5):CD004714.

37. Engebretson SP, Hyman LG, Michalowicz BS, Schoenfeld ER, Gelato MC, Hou W, et al. The effect of nonsurgical periodontal therapy on hemoglobin A1c levels in persons with type 2 diabetes and chronic periodontitis: a randomized clinical trial. Jama. 2013;310(23):2523-32.

38. Li C, Lv Z, Shi Z, Zhu Y, Wu Y, Li L, et al. Periodontal therapy for the management of cardiovascular disease in patients with chronic periodontitis. Cochrane Database Syst Rev. 2014;8:Cd009197.

39. Viergever RF, Karam G, Reis A, Ghersi D. The quality of registration of clinical trials: still a problem. PLoS One. 2014;9(1):e84727.

40. Joshi N, Bissada NF, Bodner D, Maclennan GT, Narendran S, Jurevic R, et al. Association between periodontal disease and prostate-specific antigen levels in chronic prostatitis patients. J Periodontol. 2010;81(6):864-9.

41. Linden GJ, Lyons A, Scannapieco FA. Periodontal systemic associations: review of the evidence. J Periodontol. 2013;84(4 Suppl):S8-s19.

42. Chen SC, Marino V, Gronthos S, Bartold PM. Location of putative stem cells in human periodontal ligament. J Periodontal Res. 2006;41(6):547-53.

43. Lin NH, Menicanin D, Mrozik K, Gronthos S, Bartold PM. Putative stem cells in regenerating human periodontium. J Periodontal Res. 2008;43(5):514-23.

44. Kimura Y, Komaki M, Iwasaki K, Sata M, Izumi Y, Morita I. Recruitment of bone marrow-derived cells to periodontal tissue defects. Front Cell Dev Biol. 2014;2:19.

45. Aicher A, Rentsch M, Sasaki K, Ellwart JW, Fandrich F, Siebert R, et al. Nonbone marrow-derived circulating progenitor cells contribute to postnatal neovascularization following tissue ischemia. Circ Res. 2007;100(4):581-9.

46. Rennert RC, Sorkin M, Garg RK, Gurtner GC. Stem cell recruitment after injury: lessons for regenerative medicine. Regen Med. 2012;7(6):833-50.

47. Lapidot T, Kollet O. The essential roles of the chemokine SDF-1 and its receptor CXCR4 in human stem cell homing and repopulation of transplanted immune-deficient NOD/SCID and NOD/SCID/B2m(null) mice. Leukemia. 2002;16(10):1992-2003.

48. Gil-Ortega M, Garidou L, Barreau C, Maumus M, Breasson L, Tavernier G, et al. Native adipose stromal cells egress from adipose tissue in vivo: evidence during lymph node activation. Stem Cells. 2013;31(7):1309-20.

49. Tseng C, Kolonin MG. Proteolytic Isoforms of SPARC Induce Adipose Stromal Cell Mobilization in Obesity. Stem Cells. 2015.

50. . !!! INVALID CITATION !!!

51. Loe H. Periodontal disease. The sixth complication of diabetes mellitus. Diabetes Care. 1993;16(1):329-34.

52. Llambes F, Arias-Herrera S, Caffesse R. Relationship between diabetes and periodontal infection. World J Diabetes. 2015;6(7):927-35.

53. Guggenheimer J, Moore PA, Rossie K, Myers D, Mongelluzzo MB, Block HM, et al. Insulin-dependent diabetes mellitus and oral soft tissue pathologies. I. Prevalence and characteristics of non-candidal lesions. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2000;89(5):563-9.

54. Yu L, Tu Q, Han Q, Zhang L, Sui L, Zheng L, et al. Adiponectin regulates bone marrow mesenchymal stem cell niche through a unique signal transduction pathway: an approach for treating bone disease in diabetes. Stem Cells. 2015;33(1):240-52.

55. Segal MS, Shah R, Afzal A, Perrault CM, Chang K, Schuler A, et al. Nitric oxide cytoskeletal-induced alterations reverse the endothelial progenitor cell migratory defect associated with diabetes. Diabetes. 2006;55(1):102-9.

56. Wang A, Guilpain P, Chong BF, Chouzenoux S, Guillevin L, Du Y, et al. Dysregulated expression of CXCR4/CXCL12 in subsets of patients with systemic lupus erythematosus. Arthritis Rheum. 2010;62(11):3436-46.

57. Havens AM, Chiu E, Taba M, Wang J, Shiozawa Y, Jung Y, et al. Stromal-derived factor-1alpha (CXCL12) levels increase in periodontal disease. J Periodontol. 2008;79(5):845-53.

58. Abe D, Kubota T, Morozumi T, Shimizu T, Nakasone N, Itagaki M, et al. Altered gene expression in leukocyte transendothelial migration and cell communication pathways in periodontitis-affected gingival tissues. J Periodontal Res. 2011;46(3):345-53.

59. Song M, Heo J, Chun JY, Bae HS, Kang JW, Kang H, et al. The paracrine effects of mesenchymal stem cells stimulate the regeneration capacity of endogenous stem cells in the repair of a bladder-outlet-obstruction-induced overactive bladder. Stem Cells Dev. 2014;23(6):654-63.

60. Bernardo ME, Fibbe WE. Mesenchymal stromal cells: sensors and switchers of inflammation. Cell Stem Cell. 2013;13(4):392-402.

61. Murphy MB, Moncivais K, Caplan AI. Mesenchymal stem cells: environmentally responsive therapeutics for regenerative medicine. Exp Mol Med. 2013;45:e54.

62. Noel D, Djouad F, Bouffi C, Mrugala D, Jorgensen C. Multipotent mesenchymal stromal cells and immune tolerance. Leuk Lymphoma. 2007;48(7):1283-9.

63. Leto Barone AA, Khalifian S, Lee WP, Brandacher G. Immunomodulatory effects of adipose-derived stem cells: fact or fiction? Biomed Res Int. 2013;2013:383685.

64. Bacigalupo A, Valle M, Podesta M, Pitto A, Zocchi E, De Flora A, et al. T-cell suppression mediated by mesenchymal stem cells is deficient in patients with severe aplastic anemia. Exp Hematol. 2005;33(7):819-27.

65. Francois M, Copland IB, Yuan S, Romieu-Mourez R, Waller EK, Galipeau J. Cryopreserved mesenchymal stromal cells display impaired immunosuppressive properties as a result of heat-shock response and impaired interferon-gamma licensing. Cytotherapy. 2012;14(2):147-52.

66. Preshaw PM, Seymour RA, Heasman PA. Current concepts in periodontal pathogenesis. Dent Update. 2004;31(10):570-2, 4-8.

67. Beirne P, Worthington HV, Clarkson JE. Routine scale and polish for periodontal health in adults. Cochrane Database Syst Rev. 2007(4):CD004625.

68. Renz A, Ide M, Newton T, Robinson PG, Smith D. Psychological interventions to improve adherence to oral hygiene instructions in adults with periodontal diseases. Cochrane Database Syst Rev. 2007(2):CD005097.

69. Claffey N, Nylund K, Kiger R, Garrett S, Egelberg J. Diagnostic predictability of scores of plaque, bleeding, suppuration and probing depth for probing attachment loss. 3 1/2 years of observation following initial periodontal therapy. J Clin Periodontol. 1990;17(2):108-14.

70. Needleman IG, Worthington HV, Giedrys-Leeper E, Tucker RJ. Guided tissue regeneration for periodontal infra-bony defects. Cochrane Database Syst Rev. 2006(2):CD001724.

71. ADA, editor Combating antibiotic resistance. J Am Dent Assoc; 2004 Apr.

72. Sweeney LC, Dave J, Chambers PA, Heritage J. Antibiotic resistance in general dental practice--a cause for concern? J Antimicrob Chemother. 2004;53(4):567-76.

73. Slots J, Pallasch TJ. Dentists' role in halting antimicrobial resistance. J Dent Res. 1996;75(6):1338-41.

74. Colombo AP, Haffajee AD, Dewhirst FE, Paster BJ, Smith CM, Cugini MA, et al. Clinical and microbiological features of refractory periodontitis subjects. J Clin Periodontol. 1998;25(2):169-80.

75. Colombo AP, Sakellari D, Haffajee AD, Tanner A, Cugini MA, Socransky SS. Serum antibodies reacting with subgingival species in refractory periodontitis subjects. J Clin Periodontol. 1998;25(7):596-604.

76. Haffajee AD, Socransky SS, Dzink JL, Taubman MA, Ebersole JL, Smith DJ. Clinical, microbiological and immunological features of subjects with destructive periodontal diseases. J Clin Periodontol. 1988;15(4):240-6.

77. Hirschfeld L, Wasserman B. A long-term survey of tooth loss in 600 treated periodontal patients. J Periodontol. 1978;49(5):225-37.

78. McFall WT, Jr. Tooth loss in 100 treated patients with periodontal disease. A long-term study. J Periodontol. 1982;53(9):539-49.

79. Esposito M, Grusovin MG, Papanikolaou N, Coulthard P, Worthington HV. Enamel matrix derivative (Emdogain(R)) for periodontal tissue regeneration in intrabony defects. Cochrane Database Syst Rev. 2009(4):CD003875.

80. Bosshardt DD, Selvig KA. Dental cementum: the dynamic tissue covering of the root. Periodontol 2000. 1997;13:41-75.

81. Monsarrat P, Vergnes JN, Nabet C, Sixou M, Snead ML, Planat-Benard V, et al. Concise Review: Mesenchymal Stromal Cells Used for Periodontal Regeneration: A Systematic Review. Stem Cells Transl Med. 2014.

82. Levac D, Colquhoun H, O'Brien K. Scoping studies: advancing the methodology. Implementation Science. 2010;5(1):69.

83. Anderson S, Allen P, Peckham S, Goodwin N. Asking the right questions: scoping studies in the commissioning of research on the organisation and delivery of health services. Health Res Policy Syst. 2008;6:7.

84. Ehrich K, Freeman GK, Richards SC, Robinson IC, Shepperd S. How to do a scoping exercise: continuity of care. Research Policy and Planning. 2002;20(1):25-9.

85. Blasco-Baque V, Serino M, Vergnes JN, Riant E, Loubieres P, Arnal JF, et al. High-fat diet induces periodontitis in mice through lipopolysaccharides (LPS) receptor signaling: protective action of estrogens. PLoS One. 2012;7(11):e48220.

86. Fawzy El-Sayed KM, Paris S, Becker ST, Neuschl M, De Buhr W, Salzer S, et al. Periodontal regeneration employing gingival margin-derived stem/progenitor cells: an animal study. J Clin Periodontol. 2012;39(9):861-70.

87. Yang H, Aprecio RM, Zhou X, Wang Q, Zhang W, Ding Y, et al. Therapeutic effect of TSG-6 engineered iPSC-derived MSCs on experimental periodontitis in rats: a pilot study. PLoS One. 2014;9(6):e100285.

88. Akita D, Morokuma M, Saito Y, Yamanaka K, Akiyama Y, Sato M, et al. Periodontal tissue regeneration by transplantation of rat adipose-derived stromal cells in combination with PLGA-based solid scaffolds. Biomed Res. 2014;35(2):91-103.

89. Bayan C, Levitt JM, Miller E, Kaplan D, Georgakoudi I. Fully automated, quantitative, noninvasive assessment of collagen fiber content and organization in thick collagen gels. J Appl Phys. 2009;105(10):102042.

90. Tobita M, Uysal CA, Guo X, Hyakusoku H, Mizuno H. Periodontal tissue regeneration by combined implantation of adipose tissue-derived stem cells and platelet-rich plasma in a canine model. Cytotherapy. 2013.

91. Roach HI. Why does bone matrix contain non-collagenous proteins? The possible roles of osteocalcin, osteonectin, osteopontin and bone sialoprotein in bone mineralisation and resorption. Cell Biol Int. 1994;18(6):617-28.

92. Giachelli CM, Steitz S. Osteopontin: a versatile regulator of inflammation and biomineralization. Matrix Biol. 2000;19(7):615-22.

93. Arzate H, Zeichner-David M, Mercado-Celis G. Cementum proteins: role in cementogenesis, biomineralization, periodontium formation and regeneration. Periodontol 2000. 2015;67(1):211-33.

94. Bosshardt DD, Stadlinger B, Terheyden H. Cell-to-cell communication--periodontal regeneration. Clin Oral Implants Res. 2015;26(3):229-39.

95. Xu FT, Li HM, Yin QS, Liang ZJ, Huang MH, Chi GY, et al. Effect of activated autologous platelet-rich plasma on proliferation and osteogenic differentiation of human adipose-derived stem cells in vitro. Am J Transl Res. 2015;7(2):257-70.

96. Valencia Mora M, Antuna Antuna S, Garcia Arranz M, Carrascal MT, Barco R. Application of adipose tissue-derived stem cells in a rat rotator cuff repair model. Injury. 2014;45 Suppl 4:S22-7.

97. Beertsen W, McCulloch CA, Sodek J. The periodontal ligament: a unique, multifunctional connective tissue. Periodontol 2000. 1997;13:20-40.

98. Abreu JG, Ketpura NI, Reversade B, De Robertis EM. Connective-tissue growth factor (CTGF) modulates cell signalling by BMP and TGF-beta. Nat Cell Biol. 2002;4(8):599-604.

99. Kemoun P, Laurencin-Dalicieux S, Rue J, Farges JC, Gennero I, Conte-Auriol F, et al. Human dental follicle cells acquire cementoblast features under stimulation by BMP-2/-7 and enamel matrix derivatives (EMD) in vitro. Cell Tissue Res. 2007;329(2):283-94.

100. Wozney JM, Rosen V, Celeste AJ, Mitsock LM, Whitters MJ, Kriz RW, et al. Novel regulators of bone formation: molecular clones and activities. Science. 1988;242(4885):1528-34.

101. Sampath TK, Maliakal JC, Hauschka PV, Jones WK, Sasak H, Tucker RF, et al. Recombinant human osteogenic protein-1 (hOP-1) induces new bone formation in vivo with a specific activity comparable with natural bovine osteogenic protein and stimulates osteoblast proliferation and differentiation in vitro. J Biol Chem. 1992;267(28):20352-62.

102. Zhang X, Guo J, Zhou Y, Wu G. The roles of bone morphogenetic proteins and their signaling in the osteogenesis of adipose-derived stem cells. Tissue Eng Part B Rev. 2014;20(1):84-92.

103. Li CJ, Madhu V, Balian G, Dighe AS, Cui Q. Cross-Talk Between VEGF and BMP-6 Pathways Accelerates Osteogenic Differentiation of Human Adipose-Derived Stem Cells. J Cell Physiol. 2015;230(11):2671-82.

104. Ebrahimian TG, Pouzoulet F, Squiban C, Buard V, Andre M, Cousin B, et al. Cell therapy based on adipose tissue-derived stromal cells promotes physiological and pathological wound healing. Arterioscler Thromb Vasc Biol. 29. United States2009. p. 503-10.

105. Rohringer S, Hofbauer P, Schneider KH, Husa AM, Feichtinger G, Peterbauer-Scherb A, et al. Mechanisms of vasculogenesis in 3D fibrin matrices mediated by the interaction of adipose-derived stem cells and endothelial cells. Angiogenesis. 2014;17(4):921-33.

106. Crisan M, Yap S, Casteilla L, Chen CW, Corselli M, Park TS, et al. A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cell. 2008;3(3):301-13.

107. Caplan AI. All MSCs are pericytes? Cell Stem Cell. 2008;3(3):229-30.

108. Nie C, Yang D, Xu J, Si Z, Jin X, Zhang J. Locally administered adipose-derived stem cells accelerate wound healing through differentiation and vasculogenesis. Cell Transplant. 2011;20(2):205-16.

109. Olea FD, Locatelli P, Hnatiuk A, De Lorenzi A, Valdivieso L, Rocha E, et al. Vascular endothelial growth factor overexpression does not enhance adipose stromal cell-induced protection on muscle damage in critical limb ischemia. Arterioscler Thromb Vasc Biol. 2015;35(1):184-8.

110. Lozito TP, Jackson WM, Nesti LJ, Tuan RS. Human mesenchymal stem cells generate a distinct pericellular zone of MMP activities via binding of MMPs and secretion of high levels of TIMPs. Matrix Biol. 2014;34:132-43.

111. Deckers MM, van Bezooijen RL, van der Horst G, Hoogendam J, van Der Bent C, Papapoulos SE, et al. Bone morphogenetic proteins stimulate angiogenesis through osteoblast-derived vascular endothelial growth factor A. Endocrinology. 2002;143(4):1545-53.

112. Peters BM, Jabra-Rizk MA, O'May GA, Costerton JW, Shirtliff ME. Polymicrobial interactions: impact on pathogenesis and human disease. Clin Microbiol Rev. 2012;25(1):193-213.

113. Falanga V. Wound healing and its impairment in the diabetic foot. Lancet. 2005;366(9498):1736-43.

114. Lipsky BA, Berendt AR, Cornia PB, Pile JC, Peters EJ, Armstrong DG, et al. 2012 Infectious Diseases Society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infections. Clin Infect Dis. 2012;54(12):e132-73.

115. Armstrong DG, Wrobel J, Robbins JM. Guest Editorial: are diabetes-related wounds and amputations worse than cancer? Int Wound J. 2007;4(4):286-7.

116. Bura A, Planat-Benard V, Bourin P, Silvestre JS, Gross F, Grolleau JL, et al. Phase I trial: the use of autologous cultured adipose-derived stroma/stem cells to treat patients with non-revascularizable critical limb ischemia. Cytotherapy. 2014;16(2):245-57.

117. Miranville A, Heeschen C, Sengenes C, Curat CA, Busse R, Bouloumie A. Improvement of postnatal neovascularization by human adipose tissue-derived stem cells. Circulation. 2004;110(3):349-55.

118. Rehman J, Traktuev D, Li J, Merfeld-Clauss S, Temm-Grove CJ, Bovenkerk JE, et al. Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. Circulation. 2004;109(10):1292-8.

119. Merfeld-Clauss S, Lupov IP, Lu H, March KL, Traktuev DO. Adipose Stromal Cell Contact with Endothelial Cells Results in Loss of Complementary Vasculogenic Activity Mediated by Induction of Activin A. Stem Cells. 2015.

120. Merfeld-Clauss S, Lupov IP, Lu H, Feng D, Compton-Craig P, March KL, et al. Adipose stromal cells differentiate along a smooth muscle lineage pathway upon endothelial cell contact via induction of activin A. Circ Res. 2014;115(9):800-9.

121. Hotamisligil GS, Erbay E. Nutrient sensing and inflammation in metabolic diseases. Nat Rev Immunol. 2008;8(12):923-34.

122. Charriere GM, Cousin B, Arnaud E, Saillan-Barreau C, Andre M, Massoudi A, et al. Macrophage characteristics of stem cells revealed by transcriptome profiling. Exp Cell Res. 2006;312(17):3205-14.

123. Saillan-Barreau C, Cousin B, Andre M, Villena P, Casteilla L, Penicaud L. Human adipose cells as candidates in defense and tissue remodeling phenomena. Biochem Biophys Res Commun. 2003;309(3):502-5.

124. Planat-Benard V, Silvestre JS, Cousin B, Andre M, Nibbelink M, Tamarat R, et al. Plasticity of human adipose lineage cells toward endothelial cells: physiological and therapeutic perspectives. Circulation. 2004;109(5):656-63.

125. Chen CY, Nace GW, Irwin PL. A 6 x 6 drop plate method for simultaneous colony counting and MPN enumeration of Campylobacter jejuni, Listeria monocytogenes, and Escherichia coli. J Microbiol Methods. 2003;55(2):475-9.

126. Baatout S, De Boever P, Mergeay M. Temperature-induced changes in bacterial physiology as determined by flow cytometry. Ann Microbiol. 2005;55:73-80.

127. Dague E, Genet G, Lachaize V, Guilbeau-Frugier C, Fauconnier J, Mias C, et al. Atomic force and electron microscopic-based study of sarcolemmal surface of living cardiomyocytes unveils unexpected mitochondrial shift in heart failure. J Mol Cell Cardiol. 2014;74:162-72.

128. Eskelinen EL, Tanaka Y, Saftig P. At the acidic edge: emerging functions for lysosomal membrane proteins. Trends Cell Biol. 2003;13(3):137-45.

129. Trouche E, Mias C, Seguelas MH, Ordener C, Cussac D, Parini A. Characterization of monoamine oxidases in mesenchymal stem cells: role in hydrogen peroxide generation and serotonin-dependent apoptosis. Stem Cells Dev. 2010;19(10):1571-8.

130. Krieger-Brauer HI, Kather H. Antagonistic effects of different members of the fibroblast and platelet-derived growth factor families on adipose conversion and NADPH-dependent H2O2 generation in 3T3 L1-cells. Biochem J. 1995;307 (Pt 2):549-56.

131. Kim JH, Kim SH, Song SY, Kim WS, Song SU, Yi T, et al. Hypoxia induces adipocyte differentiation of adipose-derived stem cells by triggering reactive oxygen species generation. Cell Biol Int. 2014;38(1):32-40.

132. Iuchi S, Weiner L. Cellular and molecular physiology of Escherichia coli in the adaptation to aerobic environments. J Biochem. 1996;120(6):1055-63.

133. Farges JC, Bellanger A, Ducret M, Aubert-Foucher E, Richard B, Alliot-Licht B, et al. Human odontoblast-like cells produce nitric oxide with antibacterial activity upon TLR2 activation. Front Physiol. 2015;6:185.

134. Kim J, Song S, Park S, Song S, Xia Y, Sung J. Primary involvement of NADPH oxidase 4 in hypoxia-induced generation of reactive oxygen species in adipose-derived stem cells. Stem Cells Dev. 2012;21(12):2212-21.

135. Bigarella CL, Liang R, Ghaffari S. Stem cells and the impact of ROS signaling. Development. 2014;141(22):4206-18.

136. Park SG, Kim JH, Xia Y, Sung JH. Generation of reactive oxygen species in adipose-derived stem cells: friend or foe? Expert Opin Ther Targets. 2011;15(11):1297-306.

137. Carriere A, Ebrahimian TG, Dehez S, Auge N, Joffre C, Andre M, et al. Preconditioning by mitochondrial reactive oxygen species improves the proangiogenic potential of adipose-derived cells-based therapy. Arterioscler Thromb Vasc Biol. 29. United States2009. p. 1093-9.

138. Higuchi M, Dusting GJ, Peshavariya H, Jiang F, Hsiao ST, Chan EC, et al. Differentiation of human adipose-derived stem cells into fat involves reactive oxygen species and Forkhead box O1 mediated upregulation of antioxidant enzymes. Stem Cells Dev. 2013;22(6):878-88.

139. Kriebel K, Biedermann A, Kreikemeyer B, Lang H. Anaerobic co-culture of mesenchymal stem cells and anaerobic pathogens - a new in vitro model system. PLoS One. 2013;8(11):e78226.

140. Saito A, Kokubu E, Inagaki S, Imamura K, Kita D, Lamont RJ, et al. Porphyromonas gingivalis entry into gingival epithelial cells modulated by Fusobacterium nucleatum is dependent on lipid rafts. Microb Pathog. 2012;53(5-6):234-42.

141. Cousin B, Munoz O, Andre M, Fontanilles AM, Dani C, Cousin JL, et al. A role for preadipocytes as macrophage-like cells. FASEB J. 1999;13(2):305-12.

142. Raicevic G, Najar M, Stamatopoulos B, De Bruyn C, Meuleman N, Bron D, et al. The source of human mesenchymal stromal cells influences their TLR profile as well as their functional properties. Cell Immunol. 2011;270(2):207-16.

143. Lombardo E, DelaRosa O, Mancheno-Corvo P, Menta R, Ramirez C, Buscher D. Toll-like receptor-mediated signaling in human adipose-derived stem cells: implications for immunogenicity and immunosuppressive potential. Tissue Eng Part A. 2009;15(7):1579-89.

144. Hwa Cho H, Bae YC, Jung JS. Role of toll-like receptors on human adipose-derived stromal cells. Stem Cells. 24. United States2006. p. 2744-52.

145. Zarember KA, Godowski PJ. Tissue expression of human Toll-like receptors and differential regulation of Toll-like receptor mRNAs in leukocytes in response to microbes, their products, and cytokines. J Immunol. 2002;168(2):554-61.

146. Pan ZK, Fisher C, Li JD, Jiang Y, Huang S, Chen LY. Bacterial LPS up-regulated TLR3 expression is critical for antiviral response in human monocytes: evidence for negative regulation by CYLD. Int Immunol. 2011;23(6):357-64.

147. Vasselon T, Detmers PA, Charron D, Haziot A. TLR2 recognizes a bacterial lipopeptide through direct binding. J Immunol. 2004;173(12):7401-5.

148. Hu YH, Zhang Y, Jiang LQ, Wang S, Lei CQ, Sun MS, et al. WDFY1 mediates TLR3/4 signaling by recruiting TRIF. EMBO Rep. 2015;16(4):447-55.

149. Wang B, Koga K, Osuga Y, Cardenas I, Izumi G, Takamura M, et al. Toll-like receptor-3 ligation-induced indoleamine 2, 3-dioxygenase expression in human trophoblasts. Endocrinology. 2011;152(12):4984-92.

150. Alcorn JF, Kolls JK. Physiology. Killer fat. Science. 2015;347(6217):26-7.

151. Zhang LJ, Guerrero-Juarez CF, Hata T, Bapat SP, Ramos R, Plikus MV, et al. Innate immunity. Dermal adipocytes protect against invasive Staphylococcus aureus skin infection. Science. 2015;347(6217):67-71.

152. Hosokawa I, Hosokawa Y, Komatsuzawa H, Goncalves RB, Karimbux N, Napimoga MH, et al. Innate immune peptide LL-37 displays distinct expression pattern from beta-defensins in inflamed gingival tissue. Clin Exp Immunol. 2006;146(2):218-25.

153. Putsep K, Carlsson G, Boman HG, Andersson M. Deficiency of antibacterial peptides in patients with morbus Kostmann: an observation study. Lancet. 2002;360(9340):1144-9.

154. de Haar SF, Hiemstra PS, van Steenbergen MT, Everts V, Beertsen W. Role of polymorphonuclear leukocyte-derived serine proteinases in defense against Actinobacillus actinomycetemcomitans. Infect Immun. 2006;74(9):5284-91.

155. Ouhara K, Komatsuzawa H, Yamada S, Shiba H, Fujiwara T, Ohara M, et al. Susceptibilities of periodontopathogenic and cariogenic bacteria to antibacterial peptides, {beta}-defensins and LL37, produced by human epithelial cells. J Antimicrob Chemother. 2005;55(6):888-96.

156. Krasnodembskaya A, Song Y, Fang X, Gupta N, Serikov V, Lee JW, et al. Antibacterial effect of human mesenchymal stem cells is mediated in part from secretion of the antimicrobial peptide LL-37. Stem Cells. 2010;28(12):2229-38.

157. Miller LS. Adipocytes armed against Staphylococcus aureus. N Engl J Med. 2015;372(14):1368-70.

158. Adams JS, Hewison M. Update in vitamin D. J Clin Endocrinol Metab. 2010;95(2):471-8.

159. Nelson CD, Reinhardt TA, Beitz DC, Lippolis JD. In vivo activation of the intracrine vitamin D pathway in innate immune cells and mammary tissue during a bacterial infection. PLoS One. 2010;5(11):e15469.

160. Fradette C, Bleau AM, Pichette V, Chauret N, Du Souich P. Hypoxia-induced down-regulation of CYP1A1/1A2 and up-regulation of CYP3A6 involves serum mediators. Br J Pharmacol. 2002;137(6):881-91.

161. Zhou S, LeBoff MS, Glowacki J. Vitamin D metabolism and action in human bone marrow stromal cells. Endocrinology. 2010;151(1):14-22.

162. Maiti A, Jiranek WA. Inhibition of Methicillin-resistant Staphylococcus aureus-induced cytokines mRNA production in human bone marrow derived mesenchymal stem cells by 1,25-dihydroxyvitamin D3. BMC Cell Biol. 2014;15:11.

163. Hurdle JG, O'Neill AJ, Chopra I, Lee RE. Targeting bacterial membrane function: an underexploited mechanism for treating persistent infections. Nat Rev Microbiol. 2011;9(1):62-75.

164. Vanhauteghem D, Janssens GP, Lauwaerts A, Sys S, Boyen F, Cox E, et al. Exposure to the proton scavenger glycine under alkaline conditions induces Escherichia coli viability loss. PLoS One. 2013;8(3):e60328.

165. Delcour AH. Outer membrane permeability and antibiotic resistance. Biochim Biophys Acta. 2009;1794(5):808-16.

166. Galkowska H, Podbielska A, Olszewski WL, Stelmach E, Luczak M, Rosinski G, et al. Epidemiology and prevalence of methicillin-resistant Staphylococcus aureus and Staphylococcus epidermidis in patients with diabetic foot ulcers: focus on the differences between species isolated from individuals with ischemic vs. neuropathic foot ulcers. Diabetes Res Clin Pract. 2009;84(2):187-93.

167. Nikaido H. Molecular basis of bacterial outer membrane permeability revisited. Microbiol Mol Biol Rev. 2003;67(4):593-656.

168. Amor KB, Breeuwer P, Verbaarschot P, Rombouts FM, Akkermans AD, De Vos WM, et al. Multiparametric flow cytometry and cell sorting for the assessment of viable, injured, and dead bifidobacterium cells during bile salt stress. Appl Environ Microbiol. 2002;68(11):5209-16.

169. Eagle H, Musselman AD. THE SLOW RECOVERY OF BACTERIA FROM THE TOXIC EFFECTS OF PENICILLIN. J Bacteriol. 1949;58(4):475-90.

170. Villena JA, Cousin B, Penicaud L, Casteilla L. Adipose tissues display differential phagocytic and microbicidal activities depending on their localization. Int J Obes Relat Metab Disord. 2001;25(9):1275-80.

171. Sung DK, Chang YS, Sung SI, Yoo HS, Ahn SY, Park WS. Antibacterial effect of mesenchymal stem cells against Escherichia coli is mediated by secretion of beta-defensin-2 via toll like receptor 4 signaling. Cell Microbiol. 2015.

172. Gupta N, Krasnodembskaya A, Kapetanaki M, Mouded M, Tan X, Serikov V, et al. Mesenchymal stem cells enhance survival and bacterial clearance in murine Escherichia coli pneumonia. Thorax. 2012;67(6):533-9.

173. Meisel R, Brockers S, Heseler K, Degistirici O, Bulle H, Woite C, et al. Human but not murine multipotent mesenchymal stromal cells exhibit broad-spectrum antimicrobial effector function mediated by indoleamine 2,3-dioxygenase. Leukemia. 2011;25(4):648-54.

174. Gupta N, Krasnodembskaya A, Kapetanaki M, Mouded M, Tan X, Serikov V, et al. Mesenchymal stem cells enhance survival and bacterial clearance in murine Escherichia coli pneumonia. Thorax. 2012.

175. Shi Y, Hu G, Su J, Li W, Chen Q, Shou P, et al. Mesenchymal stem cells: a new strategy for immunosuppression and tissue repair. Cell Res. 2010;20(5):510-8.

176. Cousin B, Andre M, Casteilla L, Penicaud L. Altered macrophage-like functions of preadipocytes in inflammation and genetic obesity. J Cell Physiol. 2001;186(3):380-6.

Conclusion générale

La thérapie cellulaire est un champ d'investigation encore jeune mais rempli de promesses. L'augmentation exponentielle du nombre d'essais cliniques et la grande variété des champs d'application illustrent l'engouement pour ces cellules. Il est appréciable de constater que les agences de santé structurent et encadrent de plus en plus leur utilisation, afin d'éviter et/ou d'enrayer la dérive mercantile de certaines sociétés privées. Le concept « cellules souches » devient un produit pharmaceutique qui tend à se spécialiser en fonction de son utilisation clinique. La réticence d'origine éthique concernant l'utilisation des cellules souches embryonnaires explique très certainement l'essor de l'intérêt grandissant pour les cellules souches « adultes ». De plus, l'obtention d'un produit cellulaire mieux caractérisé, avec une possibilité de sélection des donneurs, de certaines sous-populations cellulaires d'intérêt, pourrait expliquer que l'utilisation des CSM (cellules cultivées) soit privilégiée par rapport à la fraction hétérogène correspondante (population non cultivée). Du fait de la faible morbidité du prélèvement, de sa richesse en CSM, le tissu adipeux possède une attractivité importante.

Dans le domaine odontologique, en particulier en parodontologie, l'utilisation des CSM suscite de nombreux espoirs. En effet, les résultats peu prédictibles obtenus avec les thérapeutiques actuelles de régénération des lésions parodontales demandent de nouvelles stratégies. Compte tenu de la physiopathologie des parodontites, les propriétés des CSM permettraient un retour à l'homéostasie tissulaire, grâce au rétablissement d'un environnement favorable à l'activation et au recrutement des progéniteurs *in-situ*, et à la maîtrise des contextes inflammatoires et infectieux résiduels.

Nous avons publié une revue de la littérature qui laisse supposer que les CSM pourraient être utilisées avec succès en thérapie cellulaire du parodonte. Nous avons également montré pour la première fois une amélioration de la régénération cémentaire et de l'organisation desmodontale par la greffe d'ASC dans un modèle de parodontite induite par infusion de parodonto-pathogènes, dont la physiopathologie se rapproche plus de celle du chien et de l'homme que les modèles habituellement décrits dans la littérature. Son utilisation rend donc nos résultats plus pertinents et autorise les perspectives de l'étude des mécanismes biologiques impliqués dans ces phénomènes. Mais il convient, avant d'envisager un passage à l'Homme, de valider ces résultats chez le gros animal, le chien en particulier ; notre équipe

met donc en place un protocole de thérapie de parodontites spontanées chez le chien âgé (9-11 ans) par greffe autologue d'ASC. Ce modèle canin, original, et jamais utilisé pour la thérapie cellulaire du parodonte, présente donc un potentiel d'analyse considérable, qui pourrait nous permettre de valider que les ASC permettraient d'agir sur les trois leviers à la fois: régénération parodontale, diminution de la virulence de la flore et éventuelle exacerbation de la réaction inflammatoire, souvent de règle dans ces pathologies. L'évaluation préclinique doit être complémentaire des études *in vitro*. Nous avons démontré que les ASC possédaient une activité antibactérienne à spectre large. La poursuite de ce travail permettra de mieux décrypter la mécanistique, notamment concernant les relations microbiote/immunité/substratum, mais aussi de déterminer quelle source adipeuse (intra ou extra orale) serait la plus adaptée à la procédure, ainsi que définir les étapes du protocole opératoire.

Le concept de médecine régénérative est indissociable de la notion d'ingénierie tissulaire. Il conviendra donc également de mettre au point l'environnement porteur des cellules, qui doit être adapté aux contraintes anatomiques et architecturales ainsi qu'à la physiopathologie des lésions parodontales. En effet, la revue systématique que nous avons publiée a montré une hétérogénéité des résultats obtenus par la greffe de CSM suivant l'architecture de la lésion et le type de biomatériau porteur. Compte tenu de la grande hétérogénéité des situations lésionnelles, ce véhicule devra posséder certaines propriétés mécaniques et architecturales dédiées à ces applications, favoriser la production de matrice par les cellules, avoir différentes cinétiques de dégradation, être bioactif ou bioinerte. Dans cet objectif, nous souhaitons étudier le comportement des ASC dans un concentré plaquettaire, utilisé en cicatrisation de la muqueuse mais aussi en culture 2D des CSM. Nous avons pu adapter la conception de ce matériau porteur aux contraintes de la 3D, et nos données préliminaires montrent un excellent comportement des ASCs dans ce véhicule. D'ailleurs, l'imagerie tridimensionnelle pourrait aider à la conception de concentrés plaquettaires dont la forme est adaptée à la morphologie de la lésion tissulaire. Dans un avenir proche, l'impression tridimensionnelle serait aussi un moyen d'optimiser le couple véhicule/cellules puisque l'impression de phosphates de calcium, d'hydrogel et de cellules est désormais possible.

Parallèlement et en complément de l'étude des CSM en parodontologie, une veille attentive de la littérature scientifique est primordiale. Compte tenu de l'augmentation du volume des données dans notre société, les stratégies usuelles de revues de la littérature ne suffisent plus ; il est nécessaire d'employer de nouvelles méthodes d'exploration afin de visualiser,

interpréter l'évolution de la connaissance, et rationaliser l'utilisation des ressources. Dans le champ des CSM, cette apparente utilisation tout azimut des cellules, pose encore de nombreuses questions comme l'impact des pathologies systémiques sur la biologie et le devenir des CSM, l'utilisation d'une source allogénique ou autologue, l'âge du patient (Annexe), ou encore la « meilleure » source cellulaire pour un type de pathologie donnée. Des études comparatives entre les sources cellulaires, de type méta-analyses/méta-analyses en réseau, permettront d'établir au fur et à mesure une cartographie des potentialités par type cellulaire, que ce soit *in-vitro* (par exemple un potentiel de différenciation), *in-vivo* ou en clinique (en fonction du contexte physiopathologique de la maladie) (Figure).

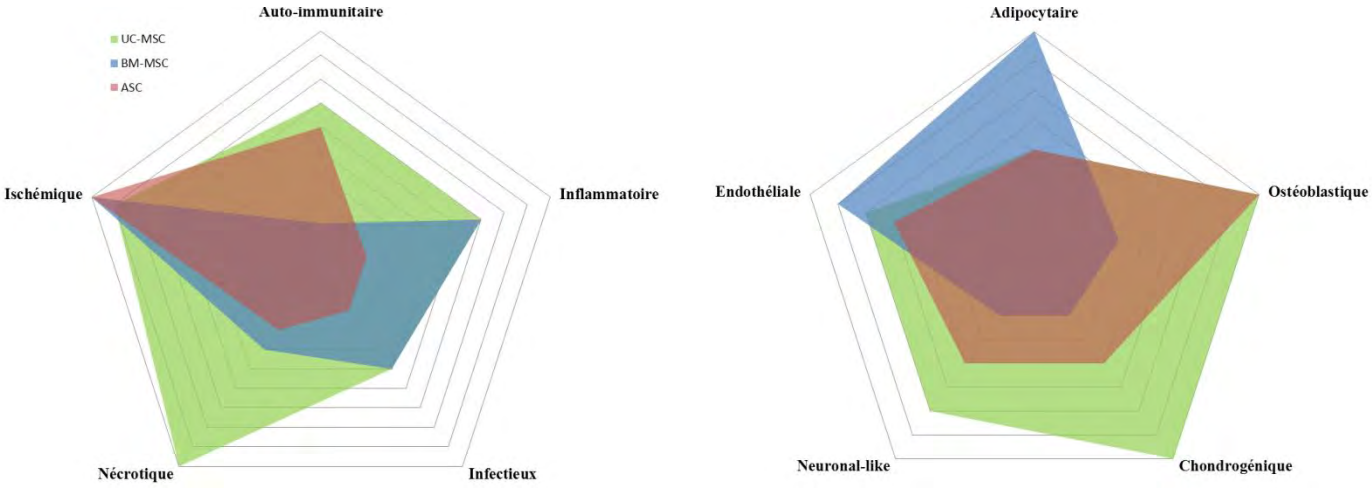


Figure : exemple de diagramme de kiviat, pouvant représenter les différences d'efficacité des différents types cellulaires, en fonction de la physiopathologie des maladies (à gauche) ou selon le potentiel de différenciation (à droite). Les données ont été choisies de manière aléatoire.

Les résultats expérimentaux que nous avons exposés dans ce travail reflètent la volonté de notre équipe de se donner les moyens de les translater avec le plus de sureté et d'efficacité chez l'homme. Ces protocoles et leurs perspectives de travail immenses nous donnent l'opportunité, non seulement de mieux comprendre la physiopathologie de cette mystérieuse maladie qu'est la parodontite, mais aussi et surtout d'espérer résoudre un véritable problème de santé publique puisque les parodontites sont des pathologies à prévalence élevée, potentiellement associées à des comorbidités graves.

La thérapie cellulaire va s'avérer déterminante pour aider à la conservation de l'organe dentaire sur l'arcade, en maintenant ainsi la qualité de vie de nos patients.

Annexes

Attestation de suivi. **Fondamentaux pour le Big Data.** *Institut Mines-Télécom [05/03/2015]*.

Attestation de suivi. **BD**2 : Des bases de Données à Big Data.** *Université Nice Sophia Antipolis [03/03/2015]*.

Abbo O, Taurand M, Monsarrat P, Raymond I, Arnaud E, de Barros S, Auriol F, Galinier P, Casteilla L, Planat-Bénard V. **Donor age dependent features of pediatric versus adult adipose mesenchymal stromal cells.** *Molecular Therapy (en cours de soumission)*.

ATTESTATION DE SUIVI AVEC SUCCÈS

Paul MONSARRAT
a suivi avec succès le MOOC*

Fondamentaux pour le Big Data
proposé par Institut Mines-Télécom
et diffusé sur la plateforme FUN
Le 05/03/2015

Les enseignants

Anne Sabourin
Enseignant-Chercheur

Joseph Salmon
Enseignant-Chercheur

Alexandre Gramfort
Enseignant-Chercheur

Ons Jelassi
Enseignante formation continue

http://www.france-universite-numerique-mooc.fr

* MOOC : cours en ligne

La présente attestation n'est pas un diplôme et ne confère pas de crédits (ECTS). Elle n'atteste pas que le participant était inscrit à/au Institut Mines-Télécom. L'identité du participant n'a pas été vérifiée.

ATTESTATION DE SUIVI AVEC SUCCÈS

Paul MONSARRAT

a suivi avec succès le MOOC*

BD**2: Des Bases de Données à Big Data

proposé par Université Nice Sophia Antipolis

et diffusé sur la plateforme FUN

Le 03/03/2015

Les enseignants

Serge Miranda
Professeur en informatique

Fabien Gandon
Directeur de Recherche chez Inria

Benjamin Renaut
Ingénieur en informatique

http://www.france-universite-numerique-mooc.fr

* MOOC : cours en ligne

La présente attestation n'est pas un diplôme et ne confère pas de crédits (ECTS). Elle n'atteste pas que le participant était inscrit à/au Université Nice Sophia Antipolis.. L'identité du participant n'a pas été vérifiée.

Donor age dependent features of pediatric versus adult adipose mesenchymal stromal cells

Olivier Abbo[1,2,3,4,5], Marion Taurand[1,2,3,4], Paul Monsarrat[1,2,3,4,6], Isabelle Raymond[7], Emmanuelle Arnaud[1,2,3,4], Sandra de Barros[1,2,3,4], Françoise Auriol[5], Philippe Galinier[5], Louis Casteilla[1,2,3,4] and Valérie Planat-Bénard*[1,2,3,4]

[1]CNRS UMR5273 STROMALab, BP 84225, F-31 432 Toulouse cedex 4, France;

[2]Université Paul Sabatier de Toulouse, BP 84225, F-31 432 Toulouse cedex 4, France;

[3]INSERM U1031, BP 84225, F-31 432 Toulouse cedex 4, France;

[4]EFS Pyrénées – Méditerranée BP 84225, F-31 432 Toulouse cedex 4, France ;

[5]Service de chirurgie pédiatrique, hôpital des Enfants de Toulouse, 330 av. de Grande-Bretagne, 31069 Toulouse cedex 9, France.

[6]Toulouse Faculty of Dentistry - Paul Sabatier University and Toulouse University Hospital - CHU de Toulouse, France

[7]Université de Toulouse, UPS-INP-ENVT, Plateforme d'histopathologie expérimentale et comparée, Toulouse, France

*Corresponding author:

UMR 5273 UPS, CNRS, EFS, Inserm U1031, Laboratoire STROMALab, BP84225, 31432 Toulouse cedex 4, France. E-mail: valerie.planat@inserm.fr

Short title: Pediatric versus adult ASC properties

ABSTRACT

Adipose derived mesenchymal stromal cells (ASC) are currently tested in regenerative medicine to promote tissue reconstruction after injury. In autologous purpose the possible loss of therapeutic function and cell properties during aging have been questioned in adult. To date no reliable information is available concerning ASC from pediatric patients and a better knowledge is required to intend their use to clinical applications. To address this issue, subcutaneous adipose tissue was collected from 27 donors (0-1 year old) and 50 donors (1-12 years old) and compared to adult ASC. Cells from the stromal vascular fraction (SVF) and subsequent cultured ASC were tested *in vitro*. Only a higher amount in SVF cell number and ASC proliferative rate were found. Cell phenotype, CFU-F content, immunomodulation effect, adipogenic, osteoblastic and angiogenic potentials were not significantly different. *In vivo*, pediatric ASC induced an increase in microangiographic score in a mouse model of limb ischemia, even though improvement in vascular density was not significantly correlated to limb rescue. Finally mRNA analysis using microarray approach identified that only 305 genes were differentially expressed (217 down- and 88 up-regulated) in pediatric versus adult ASC, confirming that ASC from both groups of age shared very close intrinsic properties.

INTRODUCTION

Reconstructive surgery is a key feature of pediatric surgery, whatever the organ involved [1]. During the last century, the management of neonates and infants with congenital malformations or acquired disabilities has considerably improved, thanks to the development of both anesthetic and surgical techniques. However, multiple procedures are sometimes required during the child growth to achieve an acceptable, stable and functional outcome. As an example, the management of labio-alveolar cleft requires a long term follow up after the initial surgical procedure. Healing complications such as cutaneous scar retraction, secondary acquired palatal fistulae and other orthodontic abnormalities have to be detected rapidly in order to ensure their early correction. To overcome these limits, alternative therapies have been proposed to better restore initial anatomical and functional properties of the wounded tissue, and then avoid subsequent procedures. Stem-cell based therapy has been pointed out as a good candidate to reach this purpose [1].

Adipose mesenchymal stem/stromal cells (ASC) are well known within the field of reconstructive surgery, with lots of preclinical and now clinical applications [2, 3]. Due to specific properties, such as differentiation potential into different tissue lineages, secretion and immunomodulation characteristics [4], their abundance, with little invasive access and the absence of ethical issue, they have been widely studied in adults [5]. However, to date very few information concerning pediatric ASC properties have been reported and are insufficient to consider their clinical use in children. Maiorana et al. first reported the isolation and characterization of ASC from omental AT in 13 children (6.5 ± 4.3 years old) and 2 newborns describing their adipogenic differentiation potential and immunophenotype [6]. Guasti et al. focused on the plasticity of pediatric ASC that express pluripotency markers such as c-Myc, OCT4, Nanog, KLF4, and DNMT3B but not Sox2 and their multipotency, as pediatric ASC also express many lineage markers and differentiate into adipose, chondrogenic, osteoblastic

and neuronal cell types [7]. Due to such skeletogenic differentiation potential of ASC from the 16 children tested they concluded that adipose tissue could be a potential source of stem cells for cartilagenous or bone replacement. Wu et al. performed the only comparative study in infant (age < 1 year), adult and elderly ASC and concluded that infant ASC have long telomeres and exhibit enhanced angiogenic and osteogenic capabilities, based on 4 samples [8]. Moreover, the *in vivo* therapeutic potential of children ASC has never been evaluated. If the goal of an ideal therapy is not to repair but to regenerate a limb or organ, a more extent characterization of cells from younger donors is required as well as efficacy data in model.

The aim of our study was therefore to assess the potentiality of children ASC obtained from 27 donors from Group 0-1 year old and 50 donors from Group 1-12 years old to study sampling and phenotyping of the crude stromal vascular fraction (SVF) of adipose tissue, culture, differentiation, immunomodulation, gene expression profile and *in vivo* beneficial effect in ischemic tissue, depending on the age of the donor.

RESULTS

Pediatric adipose tissue cell properties

The efficiency of adipose tissue cells isolation from pediatric samples was compared to the conventional procedure validated for adult ASC [2]. SVF cell number obtained from children of Group 0-1 was significantly higher than Group 1-12 or adults ($1.59 \pm 0.15 \times 10^6$ compare to $0.66 \pm 0.06 \times 10^6$ and $0.69 \pm 0.04 \times 10^6$ respectively). Similar amount of SVF cells were obtained with Group 1-12 and adults samples (Fig. 1a). The amount of progenitor cells in SVF cells was assessed by the number of CFU-F. No significant difference was noticed between groups (Fig. 1b). The phenotype of SVF cells was homogeneous between pediatric donors from 0 to 12 years old. When compared to adults, no major difference was observed

(Fig. 1c). Surface markers analysis just pointed out that the amount of endothelial cells ($CD34^+$ $CD31^+$ $CD45^-$) was higher (p=0.038) in pediatric SVF cells whereas lymphocyte T tented to slightly more important in adult samples (p=0.307 for LT $CD3^+$ and p=0.290 for LT $CD4^+$). Considering more specifically the ASC subpopulation, the yield of ASC obtained in primary culture (ASC P0) was similar between Group 0-1 and Group 1-12 but Group 0-1 was significantly higher compared to adults with an expansion factor of 4.77 (Group 0-1), 4.69 (Group1-12) and 3.87 (Adult) (Fig. 1d). The amount of CFU-F obtained from ASC was very close in the three groups (13.6% ± 3, 12.1% ± 4.5 and 12.9 % ± 3.8 respectively, Fig. 1e).

Pediatric ASC differentiation potentials

Adult ASC are multipotent and known to be able to differentiate into adipogenic, osteogenic and chondrogenic lineages and to support vascular network formation *in vitro* [5]. In the present study, the adipogenic and osteogenic potentials of pediatric ASC from both groups were found to be similar as adult ASC (Fig. 2a,b). Triglyceride accumulation under adipogenic induction appeared to be 2-times higher in ASC Group 0-1 (Fig. 2a) and mineralization under osteogenic induction was higher in ASC 1-12 (Fig. 2b), however these differences were not statistically significant from adult ASC. When assessing specific differentiation marker expression no difference was observed. Concerning the level of expression of endothelial markers under VEGF stimulation, there was no significant difference between the 3 groups of ASC, as well as for their capacity to support CD31vascular network formation *in vitro* (Fig. 2c).

Immunosuppressive properties

Immunomodulatory properties of adult ASC have been previously described *in vitro* [9] and were challenged *in vivo* in Graft versus Host Disease [10]. The immunosuppressive

activity of ASC towards T lymphocyte proliferation was estimated *in vitro* and results highlighted that pediatric ASC displayed similar inhibitory activity to adult ASC (Fig. 3).

Effect of *in vitro* replicative stress on pediatric ASC

In vitro cell expansion might be associated with replicative senescence and stress that may generate mutations, chromosomal abnormalities and many other cell defects not suitable with the perspective of cell administration for therapeutic purpose. Some data are available concerning adult ASC [11] but this point has never been addressed with pediatric ASC. As cell transformation has been associated with an increase in telomerase activity that shortens telomere, and a loss in proliferative activity control, the risks induced by the cell culture process was evaluated. We first calculated the cumulated doubling time over 10 passages showing that ASC from Group 0-1 cycled more rapidly than ASC from Group 1-12 ($p=0.0025$) and adult ($p=0.036$). Doubling time corresponded to 26.5 ± 1.2 days for Group 0-1, 37.8 ± 2.3 days for Group 1-12 and 33.4 ± 0.9 days for the adult group (Fig. 4, first panel). We also assessed the level of expression of the main molecules of the replicative senescence checkpoint (p53, p21, p16, MDM2 and pRB), hTERT, Myc for proliferative activity and Oct4, Sox2 for cell stemness status during 10 passages, each performed at the subconfluence cell density. The result showed that except for p16, which expression was increasing with passages, the expression of all the other factors stayed stable or even tented to decrease in long term culture (Fig. 4).

***In vivo* angiogenic potential of pediatric ASC in a mouse model of limb ischemia**

To investigate the *in vivo* potential of pediatric ASC, we used the previously described mouse model of limb ischemia where pediatric ASC P1 were injected [11,14]. First, we assessed the percentage of hind limb conservation with or without *in situ* injection of pediatric

ASC (Fig. 5a). No significant difference was found between the two groups with respectively 66,6% and 73.3% of limbs conservation in animals treated with ASC from Group 0-1 and 1-12, versus 70.1% in untreated animals. When ischemic lesions were observed in the conserved limb (from distal finger necrosis to partial limb amputation), we compared the length of the remaining hind limb (expressed as the ratio of the full non-ischemic length) (Fig. 5b). The remaining ischemic limb was always shorter in the control group (0.63±0.05) compared to the ASC injected groups (0.83±0.08 and 0.68±0.07 with ASC from Group 1-0 and 1-12 respectively) without reaching significant difference. When no macroscopic lesion occurred, we compared the superficial blood flow recovery. No difference was observed comparing the injected and non-injected groups, whatever the day tested after femoral artery ligation (Fig. 5c). In both groups, the blood flow similarly increased from day 0 to day 14. Finally, we assessed the microvascularization development in response to ischemia in both groups by measuring the pixel density on microangiography imaging (Fig. 5d). The vascular density was significantly improved when pediatric ASC were injected; 0.87 ± 0.02 for group 0-1 (n=8) and 0.91 ± 0.03 for group 1-12 (n=15) compare to control. Taken together pediatric ASC from both groups significantly improve neovascularization in ischemic tissue reaching 0.9 ± 0.02 (n=23) versus 0.84 ± 0.01 in control (n=23, p=0.0184). To sum up, no strong benefit was associated with pediatric ASC administration at the macroscopic level. Only an improvement in mircrovessels density was observed in the ischemic muscle, suggesting that the angiogenic potential is somehow sustained from pediatric to adult ASC.

Global gene expression profile between pediatric versus adult ASC

Due to the modest changes noticed in ASC during aging we developed a global approach to focus attention on the possible significant variations in the gene expression program. Gene expression pattern for a total of 33,297 genes was then assessed in microarray

and only 217 and 88 were significantly down- and up-regulated in pediatric compared to adult ASC (p-value < 0.05 with FC > log2, Fig. 6a). The complete list of genes sorted by log fold change, were detailed in the supplementary Tables S1 and S2. The clustering of pediatric and adult ASC into two separated groups was obtained as expected, even though the P1 donor was closer to adults according to his the gene expression profile (Fig. 6a). Within each group a quite similar profile was obtained. Two KEGG pathways mapping molecular interaction and reaction between genes down-regulated in children were identified (supplementary Table S3); the NOD-like receptor pathway (p=0.04) and genes about circadian rhythm (p=0.05). One pathway was found up regulated and was related to the aminoacyl-tRNA biosynthesis pathway (p=0.008). Differentially expressed gene sets could also be classified into biological processes (Fig. 6b) such as biosynthesis (clustered in GO terms “tRNA aminoacylation for protein translation”, “amino acid activation” and “L-serine metabolic process”) that were up-regulated in pediatric versus adult ASC. On the contrary, several genes about immune response and stress cellular response were down-regulated (e.g. clustered in GO terms “positive regulation of response to external stimulus”, “response to lipopolysaccharide”, “inflammatory response” or “response to wounding”) in children. Up- and down-regulated genes involved in each identified GO term, were detailed in Fig. 6b. Taken together this analysis confirmed that only minor differences could be highlighted between pediatric and adult ASC according to their gene expression profile.

DISCUSSION

Studies reporting the potential of human ASC in various experimental models mainly focused on the use of ASC from adult donors (even elderly) [12] but information about pediatric cells are still lacking with only three previous studies [6-8]. In accordance with the

unique comparative study [7] our data lead to the conclusion that pediatric ASC have similar *in vitro* properties than adult ASC concerning extraction, *in vitro* expansion and immunosuppressive effect, based on 77 samples testing. Regarding to functional assays and gene expression we also found that ASC from each group of donor display similar differentiation potentials into bone and adipose cells. This result is in agreement with the study of Guasti et al. [7] that have been enthusiastic concerning the possible use of pediatric ASC to reconstruct bone and cartilage defect or malformations. The authors also pointed out that differentiation should be strictly directed by defined medium as cells from young donors may easily engage into multiple lineages simultaneously.

In our hands pediatric ASC can express endothelial cells markers and can also support vascular network formation *in vitro* and *in vivo*. The *in vitro* angiogenic properties of pediatric ASC were similar to adult cells. This result correlates with the mRNA expression level of the angiogenic the factor VEGF-A comparable in children and adults, according to Affymetrix or RT-qPCR analyses. However this observation differs from Wu et al. findings. They report that the expression level of VEGF and FGF-2 were higher in children than in adults and elderly samples and concluded that infant ASC represent a crucial source for angiogenesis and vasculogenesis [8]. The ASC culture conditions or individual donor status should be closely compared as they may generate the observed differences.

In the mouse model of limb ischemia the beneficial effect of ASC in neo-vascularization seemed to be weaker than the effect previously reported with adult ASC [13, 14]. To clearly and definitively conclude on the *in vivo* effect, additional children samples should be tested as due to the small amount of fat obtained it was not possible in the present study to systematically performed *in vivo* experiments with pediatric samples (n=6 distinct samples tested). Nevertheless among the parameters tested the angiographic score was improved with pediatric ASC that is encouraging. When considering the effect of aging, we

reported that ASC from senior donor (>50 years old) showed a marked decrease in angiogenic potentials *in vitro* and *in vivo* as well, compared to ASC from 20-35 years old adults [13]. Taken together, this may suggest that pediatric and adult ASC display angiogenic properties that seem to be more efficient in adult cells but are severely impaired during aging.

In order to identify a possible specific signature in gene expression or representative markers of pediatric ASC compare to adult ASC, microarray experiments were performed. Minor changes in genome-wide expression were highlighted as genes differentially expressed in children represented less than 1%. Among the 88 genes up-regulated in children some are related to Y chromosome due to the fact that the pediatric cells analyzed came from boys whereas adult were essentially women. The aminoacyl tRNA synthesis pathway up regulation probably reflects an elevated rate of protein synthesis in children. To the opposite genes related to inflammatory process including cytokines and chemokine expression, defense and wounding responses are down-regulated in children, suggesting that immune responses were less mobilized. One major difference that distinguishes adult from pediatric donors is that adult adipose tissue is obtained from formerly obese donors that may have developed a sustained local inflammation signals in their adipose tissue. It is described that obese environment alters *in vitro* properties of ASC [15]. The loss of tissue homeostasis during obesity can then affect ASC biology. These findings in addition to our present results suggest that this *in situ* conditioning seems to be maintained beyond the culture process. Accordingly, ASC from morbid obesity patients are demonstrated to secrete higher concentrations of IL-8 and IL-6 in the culture medium compared to control [16]. Both cytokines expression are found significantly down regulated in children (-2.49 and -1.95 log fold change respectively) suggesting that their high level in adult may be linked to a disorder in their metabolic status. To confirm this hypothesis, adult lean donors could be compared to our adult group.

In conclusion, our study reports that pediatric ASC share similar *in vitro* properties than adult ASC. Samples collected from the youngest donors just have a slightly higher proliferation rate than older ones. Angiogenic properties are maintained in both groups of donors even though pediatric ASC appeared to be less effective *in vivo* than previously reported with adult ASC in ischemic limb. Further investigations are then required to better explore the functional properties of pediatric ASC to confirm this difference in efficacy and to determine if a pre-conditioning of pediatric ASC before administration may be of interest to enhance their therapeutic benefit.

MATERIALS AND METHODS

Adipose Tissue Sample Collection

Pediatric subcutaneous adipose tissue (1 g) was obtained from donors aged from 0 to 1 year (Group 0-1, 0.3±0.5 year old, n=27) and from 1 to 12 years (Group 1-12, 6.6±6.1 years old, n=50) undergoing elective inguinal surgeries (inguinal hernia repair and orchidopexy). The investigation was approved by the local ethical committee (ASChild1 n°08-082-03 and ASChild2 n°11-228-02), national agency (CPP-ID-RCB n°2008-A01469-46 and n°2011-A01469-32) and written informed consent from all parents. Adult subcutaneous adipose tissue (10 g) was obtained from donors undergoing elective abdominal dermolipectomy (age 20-35 years, BMI < 28). No objection certificate was obtained according to the bioethic law no. 2004-800 of August 6, 2004.

ASC Isolation and Culture

Adipose tissue samples were digested in α-MEM medium (Invitrogen, Carlsbad, CA) supplemented with 0.4 U/ml NB4 collagenase (Serva electrophoresis, Heidelberg, Germany)

for 45 min at 37°C under agitation. Cellular suspension was filtrated through 25 µm nylon membrane and centrifuged at 600g for 8 min to separate floating mature adipocytes from stromal-vascular fraction (SVF). SVF was incubated in erythrocyte lysis buffer (ammonium chloride solution, StemCell Technologies, Vancouver, Canada) for 5 minutes at 4°C and washed in PBS. SVF cells were resuspended in culture medium α-MEM for viability and cell numeration (Thoma hemocytometer).

SVF cells were then seeded at 4000 cells/cm^2 in flasks treated for cell culture (TPP, D. Dutscher, Brumath, France) in ASC expansion medium which consisted of α-MEM supplemented with 2% human plasma enriched with human platelet growth factors (PGFEP, EFS-PM Toulouse), 1 U/ml heparin Choay (Sanofi Aventis, France), 0.25 µg/ml amphotericin, 100 µg/ml streptomycin and 100 U/ml penicillin (Invitrogen). Cells were incubated at 37 °C under 5% CO_2 and the medium was changed twice a week. After 7 days, ASC were harvested with trypsin-EDTA (LifeTechnologies, Saint Aubin, France). The number of viable cells was determined using Trypan blue exclusion on a Countess cell counter. Cells were then used (ASC P0) or plated at a density of 2000 cells/cm^2 and cultured until use at passage P1 (ASC P1).

Colony Forming Units-Fibroblasts Assay

Freshly prepared SVF cells or ASC P0 were seeded in 25cm^2 flasks at 16 cells/cm^2 in ASC expansion medium. The medium was renewed every 2 or 3 days. The cultures were ended at day 14 for ASC P0 and day 10 for ASC P1. The flasks were stained with the kit RAL stainer MCDh (RAL Diagnostics, Martillac, France) to score the fibroblast colonies under an optical microscope.

Cell Phenotyping

SVF cells were incubated with PBS supplemented with FcR Block reagent (Miltenyi Biotec, Bergish Gladbach, Germany). Sextuplet staining were performed by incubating cells for 30 min at 4°C with the following conjugated primary antibodies or appropriated IgG isotype controls: Lin-FITC, CD166-PE, CD34-PerCP, CD38-PE-Cy^7, CD117-APC, CD31-FITC, CD73-PE, CD117-PE-Cy7, CD36-APC, HLA-DR-FITC, CD14-PerCP, CD11b-PE-Cy^7, CD15-APC, CD56-PE, CD19-PerCP, CD3-PE-Cy^7, CD11b-APC, TCRγδ, CD45-PerCP, CD4-PE-Cy7, CD8-APC (BD Biosciences, San Jose, CA), CD20-FITC (BioLegend, San Diego, CA), CD1a-PE (Beckman), CD45-APC-vio770 and CD-3 APC-vio770 (Miltenyi Biotec). DAPI was used for viability control. Cells were analysed on LSRFortessa^{TM} X20 (BD Bioscience, Mountain View, CA). Data acquisition was performed with FACS Diva software and analysis with Kaluza software (Beckman-Coulter, Roissy CDG, France).

Adipogenic Differentiation

Cells were plated at 4000 cells/cm^2 in 12-well tissue-culture plates (Falcon). Subconfluent ASC were induced for 3 days in adipogenic differentiation medium which consisted in ASC expansion medium supplemented with 1 µmol/l dexamethasone, 5µg/ml insulin, 1 µM rosiglitazone, 450 µM IBMX and 60 µM indomethacin (all Sigma-Aldrich, St Louis, MO). Subsequently, IBMX was removed from the medium and differentiation was extended during 11 days. The medium was changed every 3 days. The extent of differentiation was noted by observation of multilocular refringent droplets in the induced cells and by staining of neutral lipids by Oil red-O (Sigma-Aldrich) and was compared to control cells (no differentiation induction). Cellular triglyceride (TG) content was measured with a commercial test (Triglycerides Enzymatique PAP 150, Biomerieux, Marcy-l'Etoile, France). The protein content was determined using the DC Protein Assay Kit (BioRad, Marne

la Coquette, France). Differentiation quantification was evaluated by calculating the ratio of TG per total protein content.

Osteogenic Differentiation

Cells were plated at 4000 cells/cm^2 in 12-well tissue-culture plates (Falcon) and cultured until 70-80% of confluence. ASC were then cultured for 21 days in osteogenic medium which consisted in ASC expansion medium first supplemented with 0.1 µM dexamethasone, 50 µM ascorbic acid and 3 mM NaH_2PO_4 (all Sigma-Aldrich) for 14 days and then with 0.1 µM dexamethasone, 50 µM ascorbic acid and 10mM β-glycerophosphate (all Sigma-Aldrich) until day 21. The medium was changed every 3 days. Mineralization was revealed by staining calcium-rich deposits with Alizarin red (Sigma-Aldrich) and compared to control cell (no differentiation induction). Alizarin red quantity (µg) was assessed at day 0 and day 21 by addition of 10% acetic acid to the stained culture dishes and measurement of the optical density at 405 nm with a spectrophotometer. For mRNA study, the differentiation medium consisted in ASC expansion medium supplemented with 10 mM β-glycerophosphate, 50 µM ascorbic acid and 50ng/ml BMP4 (R&D systems, Minneapolis, MN, USA).

Endothelial Differentiation

SVF cells were plated at 100,000 cells/cm^2 in gelatin-coated 48-well tissue culture plates (Costar) and cultured for 10 days in ASC expansion medium supplemented or not with 10 ng/ml VEGF (Sigma-Aldrich). The medium was replaced every 3 days. Vascular tube formation was assessed by CD31 (Dako, Trappes, France) immunostaining. CD31-positive extension lengths were measured using the Elements AR 3.0 image analyzer software (Nikon, Champigny sur Marne, France).

Immunosuppression Assay

$5x10^4$ ASC were co-cultured with 10^5 $CD3^+$ T cells isolated from PBMCs (T-cell purification kit Miltenyi Biotec), in RPMI medium supplemented with 10% FBS (Hyclone, Thermo Scientific, Villebon/Yvette, France), 0.25 µg/ml amphotericin, 100 µg/ml streptomycin and 100 U/ml penicillin (Invitrogen). Before the co-cultures, $CD3^+$ T cells were labeled with 5-(and-6)-carboxyfluorescein diacetate, succinimidyl ester (CFSE) (Life Technologies) as fluorescent cell-tracing reagent. T cells were then activated with CD3/CD28 coated beads (Life Technologies). After 5 days, all cells were recovered, and T cells were stained with fluorochrome coupled anti-CD3 and anti-CD45 antibodies (Miltenyi Biotec). The proportion of cycling $CD3^+CD45^+$ T cells was quantified by fluorescence decrease of CFSE in comparison to both uncycling cells (non-stimulated T cells) and stimulated T cells cultured without ASC. Analysis involved use of the Cyan™ flow cytometer (Beckman Coulter, Villepinte, France) and Kaluza™ software. The percentage of $CD3^+$ T lymphocytes proliferating during the co-cultures were calculated in comparison to stimulated T lymphocytes without ASC as control to set the 100% of cycling cells.

Microarray Transcriptome Hybridization

After extraction, the RNAs were controlled using Nanodrop ND-1000 and Bioanalyzer 2100 Expert from Agilent. The resulting RNA were then subjected to reverse transcription using random hexamers tagged with a T7 promoter sequence followed by second-strand cDNA synthesis using a DNA polymerase (GeneChip WT cDNA Synthesis Kit; Affymetrix). The resulting double-stranded cDNA was then used for amplification of antisense cRNA and cleaned (Gene Chip Sample Cleanup Module; Affymetrix). A second cycle of cDNA synthesis was performed using random primers to reverse transcribe the cRNA into sense single stranded DNA. This DNA was fragmented, labeled, and hybridized to a human gene

chip (Human GeneChip Gene 1.0 ST Arrays; Affymetrix). Target labeling, array hybridization, washing and staining were performed upon manufacturer recommendation (GeneChip Whole Transcript [WT] Sense Target Labeling; Affymetrix). Arrays were hybridized, washed and stained (GeneChip Hybridization, Wash & Stain Kit in a GeneChip Hybridization Oven 645 and GeneChip Fluidics Station 450; Affymetrix), then scanned (GeneChip Scanner GCS3000 7G; Affymetrix) and analyzed by Command Console software.

Microarray Data Analysis

Microarray data were analyzed using tools furnished by the Bioconductor 3.1 project and R software 3.2.2 (R project). Background correcting, normalizing and calculating expression, were achieved by the Robust Multi-Array Average expression measure (RMA method from the affy package, 1.46.1). The limma package 3.24.14 was used to determine by Bayesian analysis, the differently expressed genes from 5 pediatric (P1-P5) and 5 adult (A1-A5) ASCs. Only significant genes (p-value level set at 0.05) with more than log2 1-fold up or down-regulated were kept [17-19]. Such a set of genes was annotated using oligo package 1.8.0 (pd.hugene.1.0.st.v1). Hierarchical cluster analysis was performed by Euclidean distance (average linkage clustering) and represented as a heatmap with dendrograms (gplots package 2.17.0).

All differentially expressed genes were annotated using the GO term biological processes database and the KEGG pathway database from DAVID Bioinformatics Resources 6.7 [20, 21]. Terms were considered significantly enriched at a 0.05 EASE score level, corrected by Benjamini–Hochberg procedure. REViGO [22] was then used to summarize GO terms, removing redundancy (medium similarity (0.7)).

Eighteen genes from Affymetrix and q-PCR were respectively transformed using the $2^{\Delta\Delta Ct}$ and $2^{-\Delta\Delta Ct}$ method, normalized to adults, using PUM as reference gene. Analysis of

variance model with a random effect on patient was used to detect and interpret any significant difference between the two obtained levels of expression (supplementary Fig. S1).

Real Time - quantitative PCR

Total RNA was isolated using RNAeasy minikit (Qiagen, Courtaboeuf, France) according to the manufacturer's recommendations. RNA was quantified using Nanodrop (ThermoScientific, NanoDrop products, Wilmington, USA) and 500 ng RNA was reverse-transcribed using random hexamers and Multiscribe reverse transcriptase (High Capacity cDNA Reverse Transcription kit, Thermo Fisher Scientific/Applied Biosystems, Foster, CA).

For differentiation marker expression, cDNA quantification was performed using the StepOnePlus technology and the Syber green Fast Master Mix according to manufacturer instructions (Life Technologies/Applied Biosystems). Primers PPARγ, ap2, LPL, Runx2, Osterix, Osteocalcin, PTHRX, DLX5, SNAIL, FLT1, KDR, ICAM, vWF, CD31, eNOS were used at 300nM and PUM at 100 nM.

For senescence marker expression (except for p16), quantitative PCR was performed with Sso Fast EvaGreen Supermix (Bio-Rad) and CFX96 thermal cycler (Bio-Rad). Taqman Gene expression Master kit (Life Technologies/Applied Biosystems) was used for p16 amplification. All primers PPIA, YWHAZ, p21, p53, Myc, Rb, MDM2, Oct-4, Sox2, hTERT were used at 500nM, except P16-FAM and pri Nanog (300 nM). The geometric mean of YWHAZ and PPIA was used to normalize gene expression values.

For microarray validation, 20 ng of cDNA was analyzed by real time PCR in a final volume of 20 µl using Power SYBRgreen master mix (Life Technologies/ Applied Biosystem) with the primers for VEGF, HGF, TGFbeta2, IL-8, IL-6, TNFalpha, GLUT1 (0.3 µM final). Real-time PCR assays were run on StepOne detection system instrument (Life

Technologies/ Applied Biosystem). Relative gene expression was calculated by the dCT method and normalized to PUM.

All primers sequences are detailed in supplementary Table S4.

Mouse Model of Hindlimb Ischemia

Six weeks old adult athymic nude mice (Harlan, Gannat, France) were housed in pathogen-free animal facilities (Anexplo/GenoToul, Toulouse, France). All experimental procedures were done in compliance with the French Ministry of Agriculture regulation (animal facility registration n°: MP/01/14/03/11) for animal experimentation. After anesthesia by isoflurane inhalation, a ligature was placed on the left femoral pedicle by a short inguinal incision as previously described [8]. Two hours later, 1×10^6 ASC were administrated by intramuscular injection in three different sites (gastrocnemius, gracilis, and quadriceps muscles, 20µl per injection) of the ischemic leg. For each of the six pediatric ASC samples, 4-6 mice were injected with cells (treated group, n=29) and compared with 5-6 mice (control group, n=31) undergoing similar procedure with injections of 20 µl NaCl 0.9%. Independent experiments were repeated with distinct ASC samples.

Vascular function was evaluated as the percentage of conserved ischemic limb and by the measurement of the length of the wounded leg in comparison to the non-ischemic leg of the same animal. Leg perfusion was assessed by laser Doppler imaging and limb perfusion expressed as a ratio of right ischemic to left non-ischemic leg during a 14 days follow up. At the end point, a microangiography analysis was performed under anesthesia after intracardiac injection of 1 mg/ml barium sulfate for limb vascular network imaging (Faxitron MX-20, Tucson, AR). The vessel density was expressed as a percentage of pixels per image occupied by vessels in the quantification area and compared to the non-ischemic limb of the same animal (Osirix Imaging Software, Pixmeo, Bernex, Switzerland). Quantification zone was

delineated by the ligature site on the femoral artery, the knee, the edge of the femur, and the external limit of the leg.

Statistical Analysis

Quantitative results were expressed as the mean ± SEM from independent experiments. Comparisons between groups or conditions were made with the unpaired t-test using Prism 5 software (GraphPad, San Diego, CA). Significance was defined as * $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$.

SUPPLEMENTARY MATERIAL

Figure S1: Validation of selected gene expression level between Affymetrix and RT-qPCR.

Table S1: List of genes up-regulated in pediatric ASC.

Table S2: List of genes down-regulated in pediatric ASC.

Table S3: KEEG pathways of genes differentially expressed.

Table S4: Details of the primer sequences used for qPCR.

ACKNOWLEDGEMENTS

The authors thank the surgical team of the children's hospital of Toulouse for their active participation in tissue harvesting. We thank Nathalie Marsaud (GeT-Biopuces, Toulouse Biochip Facility) for help and advices about the experimental protocol. We thank Pascale Guillou for technical assistance regarding to animal models and tissue sampling. We thank Fabian Gross from the Centre d'Investigation Clinique en Biothérapie of Toulouse Rangueil University Medical Center (CIC-BT 0511). This work was supported by Toulouse

Children Hospital (AO local 2010), the Etablissement Français du Sang (APR EFS-2011) and the ANR program SAFE 2012 (ANR-11-RPIB-0012). The authors declared no conflict of interest.

REFERENCES

1. Maghsoudlou, P., L. Urbani, and P. De Coppi, *Organ bioengineering for the newborn.* Semin Pediatr Surg, 2014. **23**(5): p. 314-23.
2. Bura, A., V. Planat-Benard, P. Bourin, J.S. Silvestre, F. Gross, J.L. Grolleau, et al., *Phase I trial: the use of autologous cultured adipose-derived stroma/stem cells to treat patients with non-revascularizable critical limb ischemia.* Cytotherapy, 2014. **16**(2): p. 245-57.
3. Pikula, M., N. Marek-Trzonkowska, A. Wardowska, A. Renkielska, and P. Trzonkowski, *Adipose tissue-derived stem cells in clinical applications.* Expert Opin Biol Ther, 2013. **13**(10): p. 1357-70.
4. Calderon, D., V. Planat-Benard, V. Bellamy, V. Vanneaux, C. Kuhn, S. Peyrard, et al., *Immune response to human embryonic stem cell-derived cardiac progenitors and adipose-derived stromal cells.* J Cell Mol Med, 2012. **16**(7): p. 1544-52.
5. Zuk, P.A., M. Zhu, P. Ashjian, D.A. De Ugarte, J.I. Huang, H. Mizuno, et al., *Human adipose tissue is a source of multipotent stem cells.* Mol Biol Cell, 2002. **13**(12): p. 4279-95.
6. Maiorana, A., A. Fierabracci, and S. Cianfarani, *Isolation and characterization of omental adipose progenitor cells in children: a potential tool to unravel the pathogenesis of metabolic syndrome.* Horm Res, 2009. **72**(6): p. 348-58.
7. Guasti, L., W. Prasongchean, G. Kleftouris, S. Mukherjee, A.J. Thrasher, N.W. Bulstrode, et al., *High plasticity of pediatric adipose tissue-derived stem cells: too much for selective skeletogenic differentiation?* Stem Cells Transl Med, 2012. **1**(5): p. 384-95.
8. Wu, W., L. Niklason, and D.M. Steinbacher, *The effect of age on human adipose-derived stem cells.* Plast Reconstr Surg, 2013. **131**(1): p. 27-37.
9. Puissant, B., C. Barreau, P. Bourin, C. Clavel, J. Corre, C. Bousquet, et al., *Immunomodulatory effect of human adipose tissue-derived adult stem cells: comparison with bone marrow mesenchymal stem cells.* Br J Haematol, 2005. **129**(1): p. 118-29.
10. Yanez, R., M.L. Lamana, J. Garcia-Castro, I. Colmenero, M. Ramirez, and J.A. Bueren, *Adipose tissue-derived mesenchymal stem cells have in vivo immunosuppressive properties applicable for the control of the graft-versus-host disease.* Stem Cells, 2006. **24**(11): p. 2582-91.
11. Bigot, N., A. Mouche, M. Preti, S. Loisel, M.L. Renoud, R. Le Guevel, et al., *Hypoxia Differentially Modulates the Genomic Stability of Clinical-Grade ADSCs and BM-MSCs in Long-Term Culture.* Stem Cells, 2015.
12. Zhu, M., E. Kohan, J. Bradley, M. Hedrick, P. Benhaim, and P. Zuk, *The effect of age on osteogenic, adipogenic and proliferative potential of female adipose-derived stem cells.* J Tissue Eng Regen Med, 2009. **3**(4): p. 290-301.

13. De Barros, S., S. Dehez, E. Arnaud, C. Barreau, A. Cazavet, G. Perez, et al., *Aging-related decrease of human ASC angiogenic potential is reversed by hypoxia preconditioning through ROS production.* Mol Ther, 2013. **21**(2): p. 399-408.
14. Planat-Benard, V., J.S. Silvestre, B. Cousin, M. Andre, M. Nibbelink, R. Tamarat, et al., *Plasticity of human adipose lineage cells toward endothelial cells: physiological and therapeutic perspectives.* Circulation, 2004. **109**(5): p. 656-63.
15. Perez, L.M., A. Bernal, B. de Lucas, N. San Martin, A. Mastrangelo, A. Garcia, et al., *Altered metabolic and stemness capacity of adipose tissue-derived stem cells from obese mouse and human.* PLoS One, 2015. **10**(4): p. e0123397.
16. Roldan, M., M. Macias-Gonzalez, R. Garcia, F.J. Tinahones, and M. Martin, *Obesity short-circuits stemness gene network in human adipose multipotent stem cells.* FASEB J, 2011. **25**(12): p. 4111-26.
17. Wagner, W., P. Horn, M. Castoldi, A. Diehlmann, S. Bork, R. Saffrich, et al., *Replicative senescence of mesenchymal stem cells: a continuous and organized process.* PLoS One, 2008. **3**(5): p. e2213.
18. Torensma, R., H.J. Prins, E. Schrama, E.T. Verwiel, A.C. Martens, H. Roelofs, et al., *The impact of cell source, culture methodology, culture location, and individual donors on gene expression profiles of bone marrow-derived and adipose-derived stromal cells.* Stem Cells Dev, 2013. **22**(7): p. 1086-96.
19. DeRisi, J.L., V.R. Iyer, and P.O. Brown, *Exploring the metabolic and genetic control of gene expression on a genomic scale.* Science, 1997. **278**(5338): p. 680-6.
20. Huang da, W., B.T. Sherman, and R.A. Lempicki, *Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources.* Nat Protoc, 2009. **4**(1): p. 44-57.
21. Huang da, W., B.T. Sherman, and R.A. Lempicki, *Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists.* Nucleic Acids Res, 2009. **37**(1): p. 1-13.
22. Supek, F., M. Bosnjak, N. Skunca, and T. Smuc, *REVIGO summarizes and visualizes long lists of gene ontology terms.* PLoS One, 2011. **6**(7): p. e21800.

FIGURE LEGENDS

Figure 1. *In vitro* properties of pediatric ASC

The amount of freshly prepared SVF cells from adipose tissue (a), n=25 Group 1-0, n=45 Group 1-12, n=85 Adult group, as well as the number of CFU-F formed (b), n=17 of each group, were compared between children and adults. SVF cells from pediatric and adult donors were analyzed for the indicated phenotypes of cell subpopulations (c), n=3 pediatric SVF, n=4 adult SVF. The amount of ASC obtained after 8 days in primary culture (ASC-P0) (d) n=26 Group 0-1, n=42 Group 1-12, n=57 Adult group, and the number of CUF-F formed (e) n=17 Group 0-1, n= 17 Group 1-12, n= 4 Adult group, were compared between children and adults. * $p \leq 0.05$ and *** $p \leq 0.001$ between indicated groups.

Figure 2. Differentiation potentials of pediatric ASC

ASC from the three different groups of donors were cultured in control (CTRL) or differentiation (Diff) medium as shown in representative images. Adipose differentiation was estimated by measuring triglyceride (TG) content and the mRNA expression level of the differentiation factors; peroxisome proliferator-activated receptor gamma (PPARγ), adipose fatty acid-binding protein (aP2) and the lipoprotein lipase (LPL) (a) n=6. Osteoblasic differentiation was estimated by measuring mineralization after Alizarin Red staining and the mRNA expression level of the transcription factors; run-related transcription factor 2 (Runx2), Osterix, parathyroid hormone 1 receptor (PTHR1), distal-less homeobox 5 (DLX5) and the zinc finger transcription regulator SNAIL (b) n=6. Angiogenic effect was estimated by measuring the branched networks after CD31immunostaining and by mRNA expression level of VEGF receptor 1 (FLT1) and 2 (KDR), intercellular adhesion molecule (ICAM), von Willebrand factor (vWF), CD31 (PECAM-1) and endothelial NO synthase (eNOS) (c), n=3-5.

Figure 3. Immonumodulation activity of pediatric ASC

CSFE labeled purified CD4+ T cells were stimulated with CD3/CD8 beads and ASC from the different groups were added or not (control) to quantify the rate of proliferating, in comparison to non-stimulated, T lymphocytes, n=8. *** $p \leq 0.001$

Figure 4. Effect of long term culture on pediatric ASC

Doubling time was calculated every 2 to 4 days and the sum over the 10 passages corresponded to cumulative doubling time (first panel) n=5 Group 0-1, n=7 Group 1-12, n=3 Adult group. Quantitative analyses of the expression of p16, p21, p53, Myc, Rb cell-cycle regulators and Oct-4, Nanaog and Sox2 stemness markers along passages, n=10. * $p \leq 0.05$

Figure 5. *In vivo* angiogenic properties of pediatric ASC

Injection of 1 x 10^6 pediatric ASC (n=6 samples) was performed in the ischemic mouse hind limb (n=4-6 per ASC sample) in comparison to non-injected control animals (n=31). Vascular function was analyzed by the percent of limb preservation (a), the length of ischemic limb (b), the quantitative evaluation of cutaneous blood flow measured by laser Doppler (c) and the angiographic score representative of the vessel density (d) expressed as the ratio of the ischemic (I) / the non-ischemic (NI) leg of each animal. * $p \leq 0.05$

Figure 6. Gene expression profiling of ASC from pediatric and adult donors

Microarray data from 5 pediatric (P1-5) and 5 adult (A1-5) ASC samples were analyzed considering that genes were significantly ($p<0.05$) differentially expressed when more than log2 1-fold was obtained and were represented in hierarchical cluster (a). The 53 genes involved in the significantly enriched GO terms about biological processes were detailed (b).

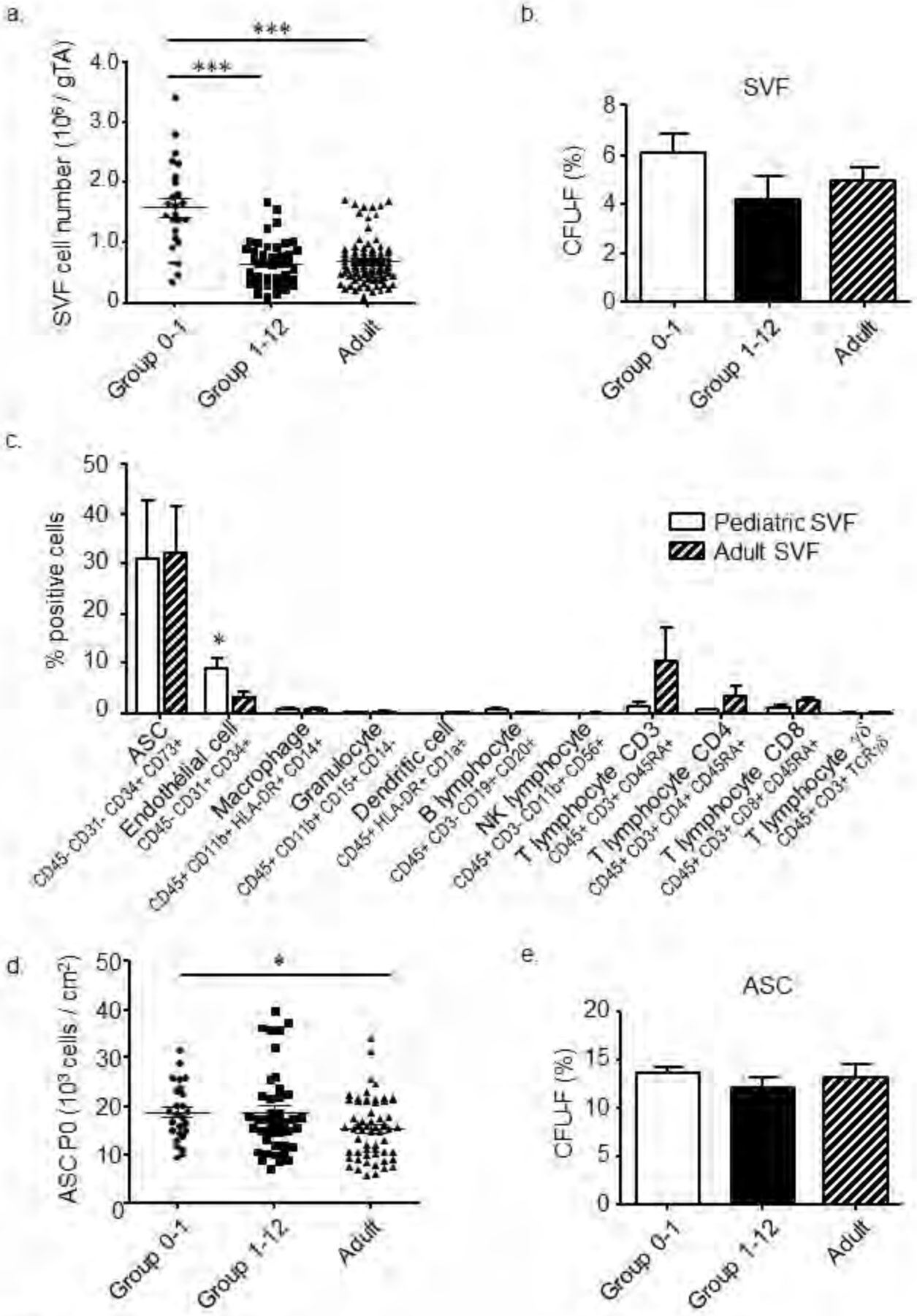

a.
SVF cell number (10⁶ / gTA)
***
***
Group 0-1
Group 1-12
Adult
b.
SVF
CFU-F (%)
Group 0-1
Group 1-12
Adult
c.
% positive cells
Pediatric SVF
Adult SVF
ASC
CD45- CD31- CD34+ CD73+
Endothelial cell
CD45- CD31+ CD34+
Macrophage
CD45+ CD11b+ HLA-DR+ CD14+
Granulocyte
CD45+ CD11b+ CD15+ CD14-
Dendritic cell
CD45+ HLA-DR+ CD1a+
B lymphocyte
CD45+ CD3- CD19+ CD20+
NK lymphocyte
CD45+ CD3- CD11b+ CD56+
T lymphocyte CD3
CD45+ CD3+ CD45RA-
T lymphocyte CD4
CD45+ CD3+ CD4+ CD45RA-
T lymphocyte CD8
CD45+ CD3+ CD8+ CD45RA-
T lymphocyte γδ
CD45+ CD3+ TCRγδ
d.
ASC P0 (10³ cells / cm²)
*
Group 0-1
Group 1-12
Adult
e.
ASC
CFU-F (%)
Group 0-1
Group 1-12
Adult

Figure 1

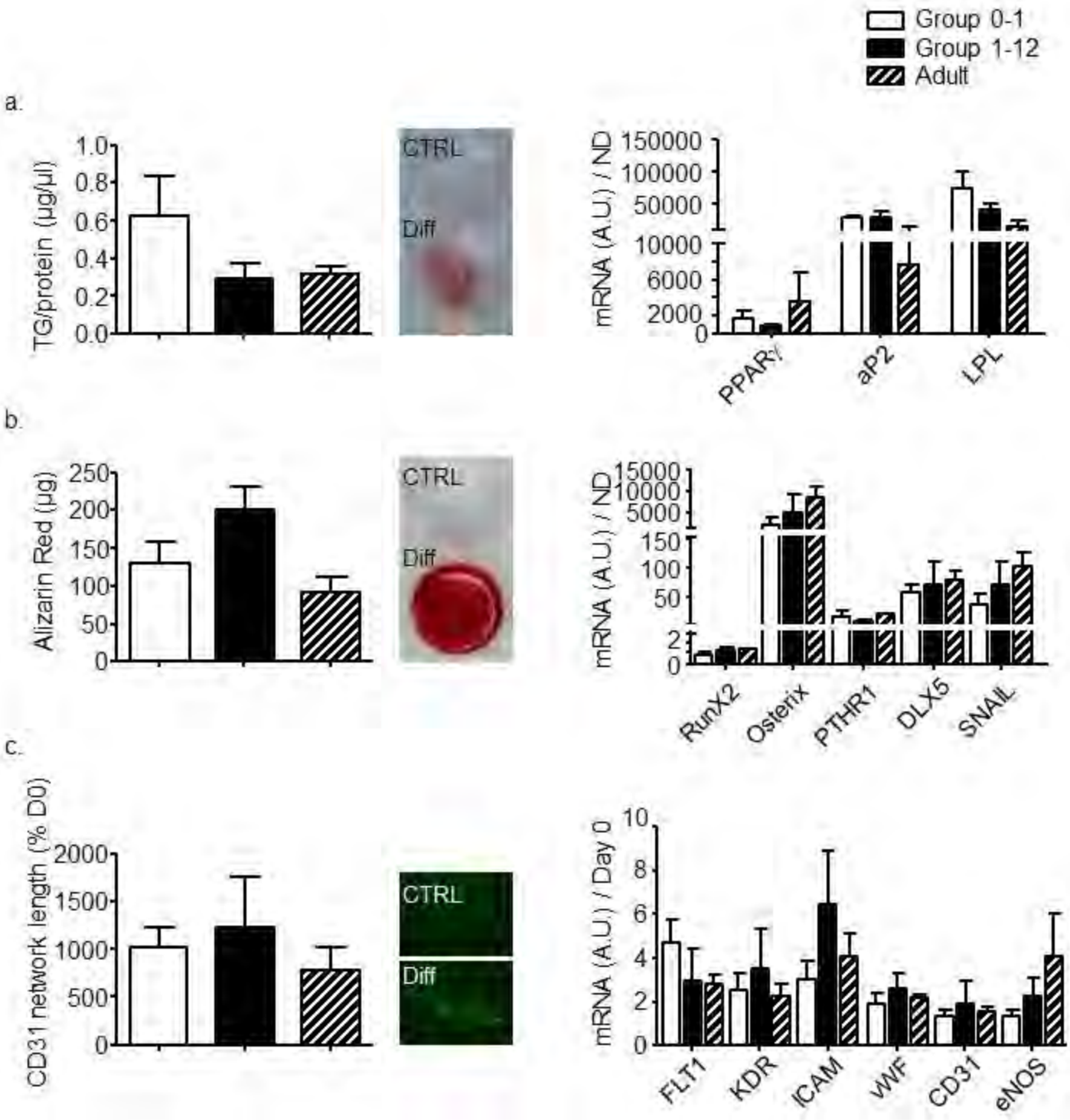

Group 0-1
Group 1-12
Adult
a.
TG/protein (µg/µl)
1.0
0.8
0.6
0.4
0.2
0.0
CTRL
Diff
mRNA (A.U.) / ND
150000
100000
50000
10000
6000
2000
0
PPARγ
aP2
LPL
b.
Alizarin Red (µg)
250
200
150
100
50
0
CTRL
Diff
mRNA (A.U.) / ND
15000
10000
5000
150
100
50
2
0
RunX2
Osterix
PTHR1
DLX5
SNAIL
c.
CD31 network length (% D0)
2000
1500
1000
500
0
CTRL
Diff
mRNA (A.U.) / Day 0
10
8
6
4
2
0
FLT1
KDR
ICAM
vWF
CD31
eNOS

Figure 2

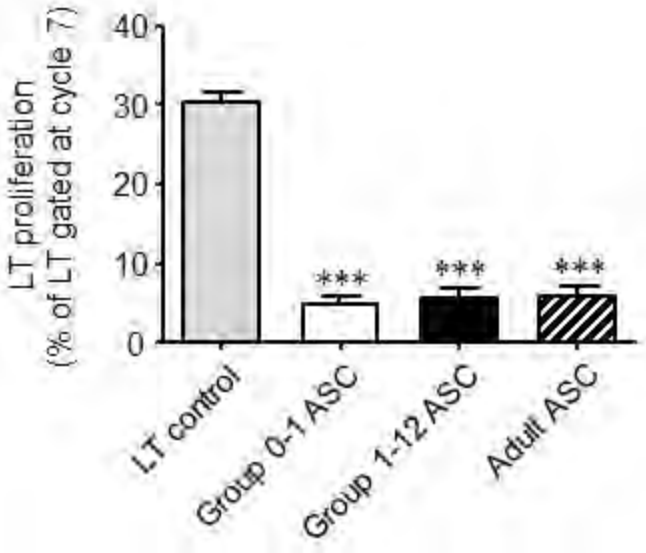

LT proliferation
(% of LT gated at cycle 7)
40
30
20
10
0
***
***
***
LT control
Group 0-1 ASC
Group 1-12 ASC
Adult ASC

Figure 3

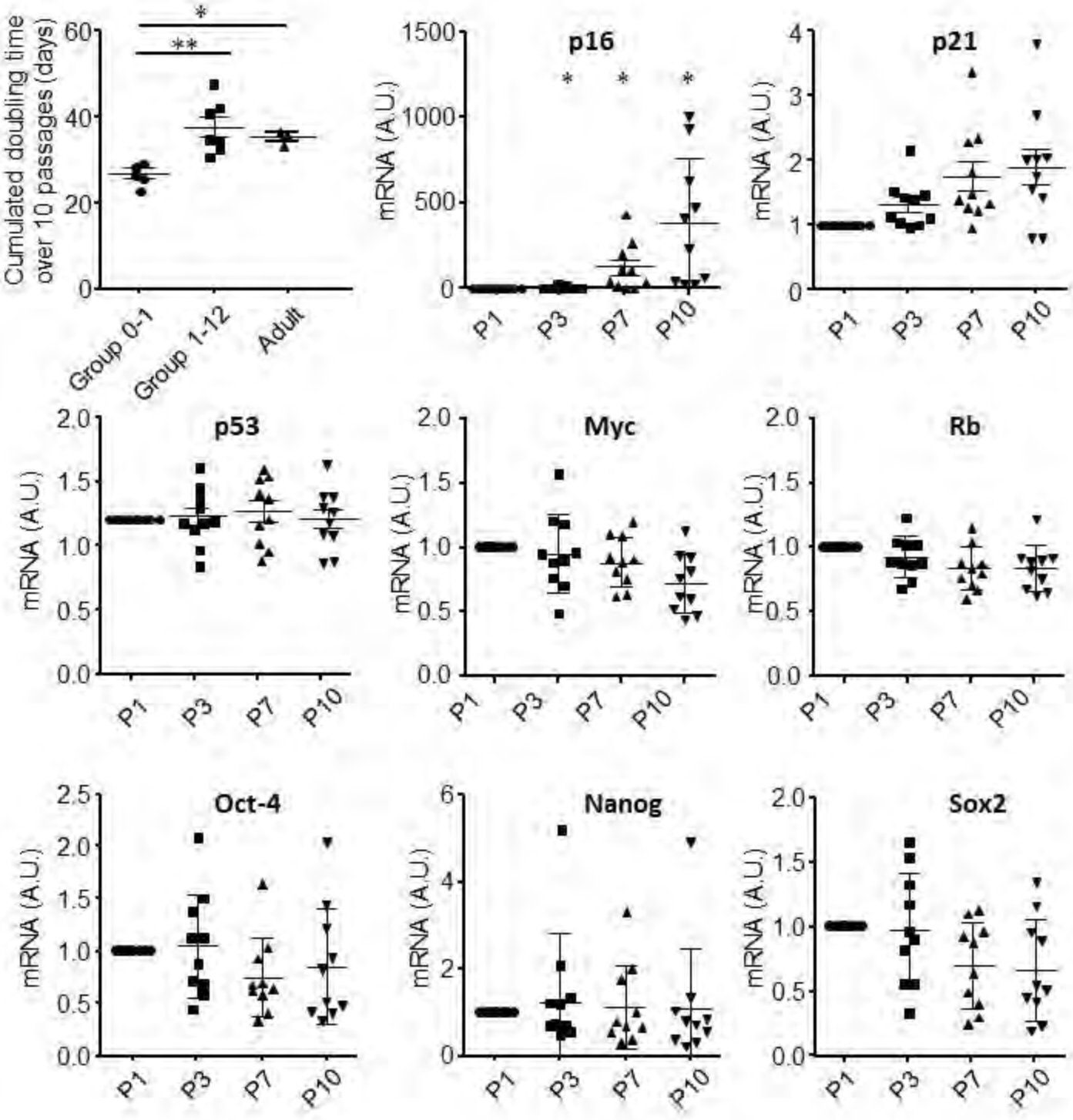

Cumulated doubling time over 10 passages (days)
60
40
20
0
*
**
Group 0-1
Group 1-12
Adult
p16
mRNA (A.U.)
1500
1000
500
0
P1
P3
P7
P10
p21
4
3
2
1
0
p53
2.0
1.5
1.0
0.5
0.0
Myc
Rb
Oct-4
2.5
Nanog
6
4
2
Sox2

Figure 4

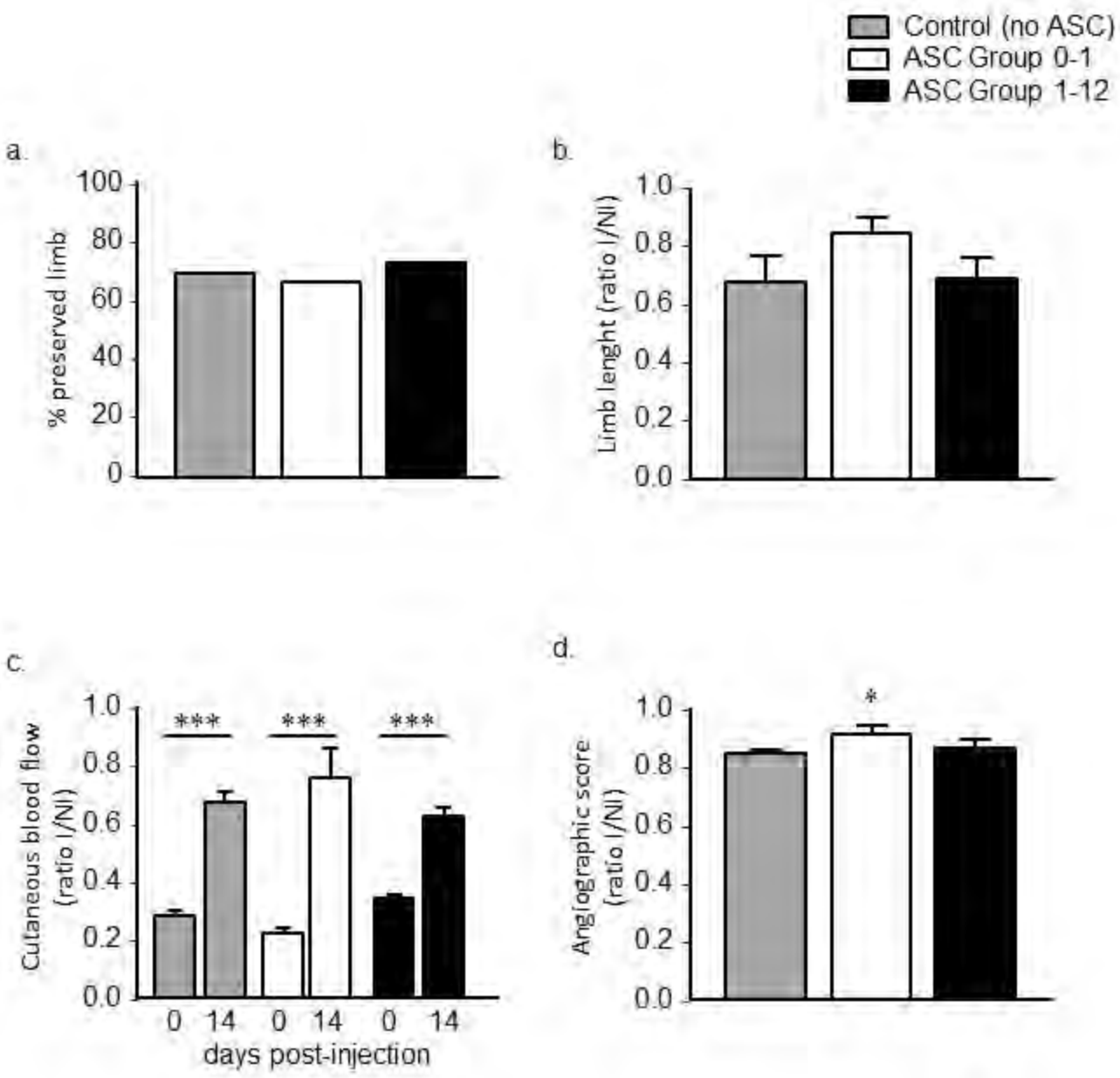

Control (no ASC)
ASC Group 0-1
ASC Group 1-12
a.
% preserved limb
100
80
60
40
20
0
b.
Limb lenght (ratio I/NI)
1.0
0.8
0.6
0.4
0.2
0.0
c.
Cutaneous blood flow (ratio I/NI)
***
***
***
0 14 0 14 0 14
days post-injection
d.
Angiographic score (ratio I/NI)
*

Figure 5

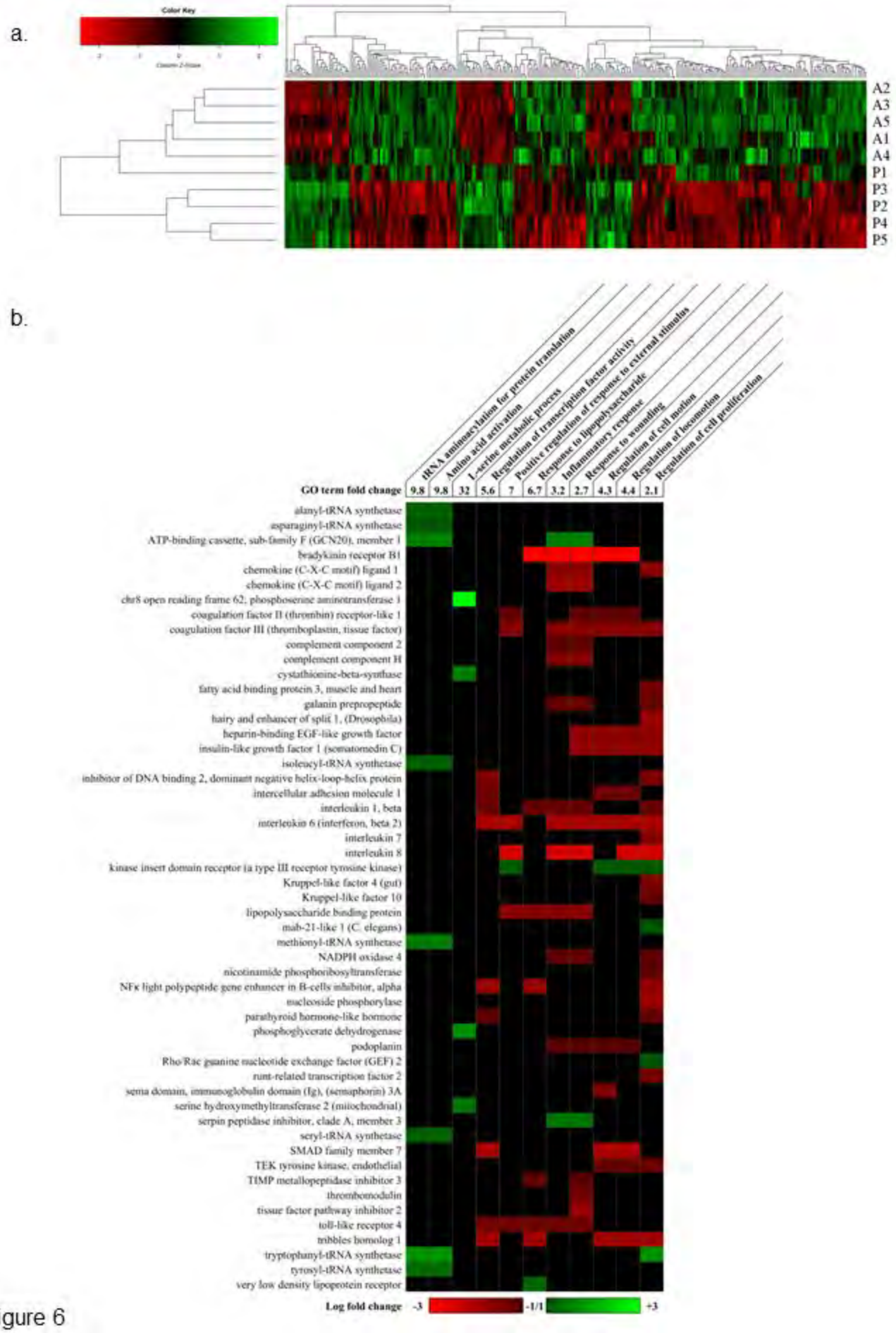
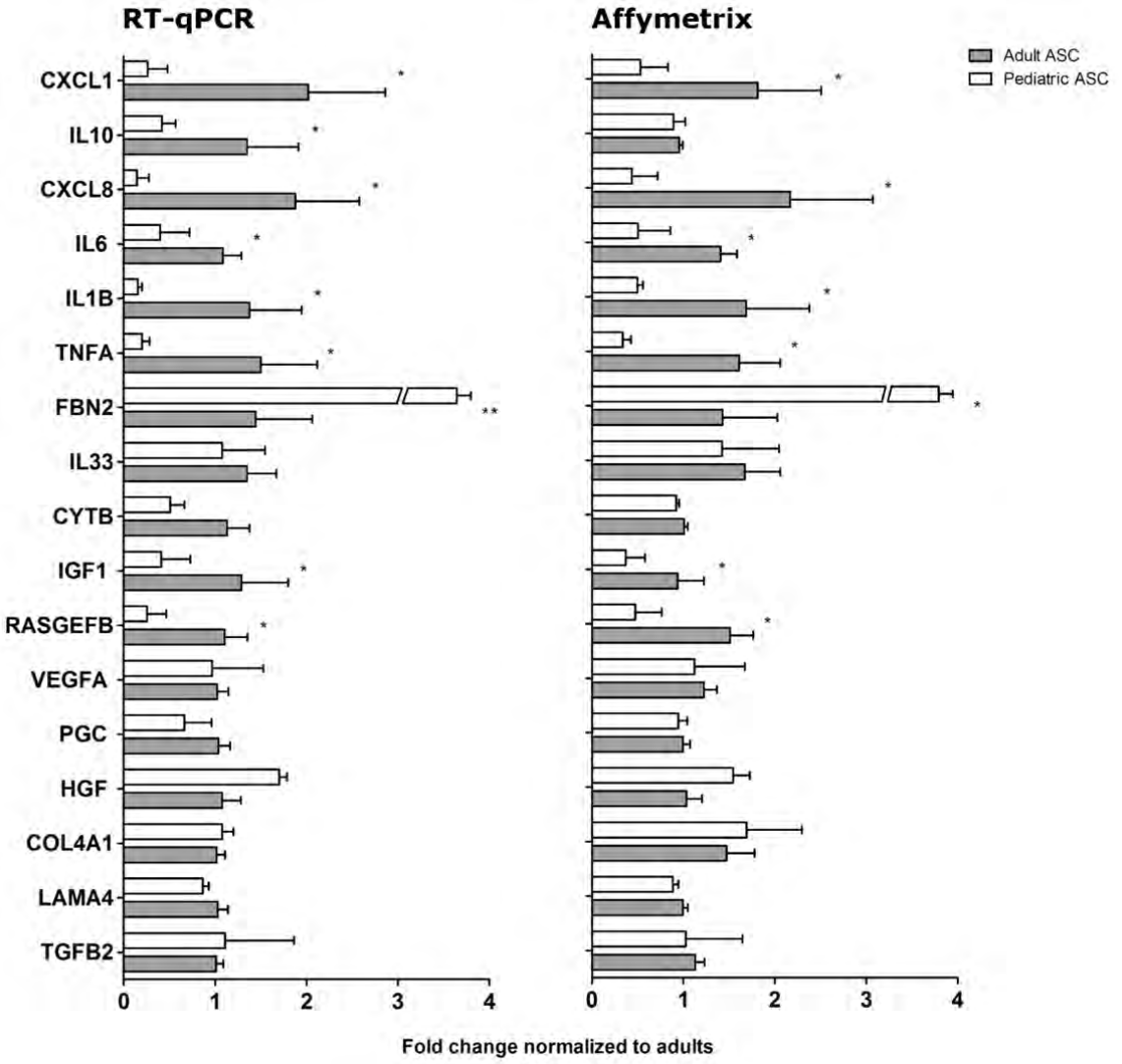

a.
Color Key
Column Z-Score
A2
A3
A5
A1
A4
P1
P3
P2
P4
P5
b.
tRNA aminoacylation for protein translation
Amino acid activation
L-serine metabolic process
Regulation of transcription factor activity
Positive regulation of response to external stimulus
Response to lipopolysaccharide
Inflammatory response
Response to wounding
Regulation of cell motion
Regulation of locomotion
Regulation of cell proliferation
GO term fold change
9.8 9.8 32 5.6 7 6.7 3.2 2.7 4.3 4.4 2.1
alanyl-tRNA synthetase
asparaginyl-tRNA synthetase
ATP-binding cassette, sub-family F (GCN20), member 1
bradykinin receptor B1
chemokine (C-X-C motif) ligand 1
chemokine (C-X-C motif) ligand 2
chr8 open reading frame 62; phosphoserine aminotransferase 1
coagulation factor II (thrombin) receptor-like 1
coagulation factor III (thromboplastin, tissue factor)
complement component 2
complement component H
cystathionine-beta-synthase
fatty acid binding protein 3, muscle and heart
galanin prepropeptide
hairy and enhancer of split 1, (Drosophila)
heparin-binding EGF-like growth factor
insulin-like growth factor 1 (somatomedin C)
isoleucyl-tRNA synthetase
inhibitor of DNA binding 2, dominant negative helix-loop-helix protein
intercellular adhesion molecule 1
interleukin 1, beta
interleukin 6 (interferon, beta 2)
interleukin 7
interleukin 8
kinase insert domain receptor (a type III receptor tyrosine kinase)
Kruppel-like factor 4 (gut)
Kruppel-like factor 10
lipopolysaccharide binding protein
mab-21-like 1 (C. elegans)
methionyl-tRNA synthetase
NADPH oxidase 4
nicotinamide phosphoribosyltransferase
NFκ light polypeptide gene enhancer in B-cells inhibitor, alpha
nucleoside phosphorylase
parathyroid hormone-like hormone
phosphoglycerate dehydrogenase
podoplanin
Rho/Rac guanine nucleotide exchange factor (GEF) 2
runt-related transcription factor 2
sema domain, immunoglobulin domain (Ig), (semaphorin) 3A
serine hydroxymethyltransferase 2 (mitochondrial)
serpin peptidase inhibitor, clade A, member 3
seryl-tRNA synthetase
SMAD family member 7
TEK tyrosine kinase, endothelial
TIMP metallopeptidase inhibitor 3
thrombomodulin
tissue factor pathway inhibitor 2
toll-like receptor 4
tribbles homolog 1
tryptophanyl-tRNA synthetase
tyrosyl-tRNA synthetase
very low density lipoprotein receptor
Log fold change
-3
-1/1
+3

Figure 6



Supplementary Figure S1: Validation of selected gene expression level between Affymetrix and RT-qPCR

Affymetrix reference	Log fold change	Description
8176375	5.94	ribosomal protein S4, Y-linked 1
8176624	5.31	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, Y-linked
8176719	3.90	eukaryotic translation initiation factor 1A, Y-linked
8156043	3.41	chromosome 8 open reading frame 62; phosphoserine aminotransferase 1
8176578	3.39	ubiquitin specific peptidase 9, Y-linked
8102800	3.26	solute carrier family 7, (cationic amino acid transporter, y+ system) member 11
8113800	3.15	fibrillin 2
8177137	2.98	ubiquitously transcribed tetratricopeptide repeat gene, Y-linked
8177232	2.96	lysine (K)-specific demethylase 5D
8176655	2.67	neuroligin 4, Y-linked
8176384	2.60	zinc finger protein, Y-linked
8176709	2.36	chromosome Y open reading frame 15B
7962516	2.34	solute carrier family 38, member 1
8176698	2.17	chromosome Y open reading frame 15A
8141150	2.12	asparagine synthetase
8162394	2.03	asporin
8103736	2.00	stimulator of chondrogenesis 1
8083415	1.74	arylacetamide deacetylase (esterase)
8176730	1.73	ribosomal protein S4, Y-linked 2
7981290	1.70	tryptophanyl-tRNA synthetase
7904433	1.63	phosphoglycerate dehydrogenase
7928308	1.63	DNA-damage-inducible transcript 4
7958784	1.62	aldehyde dehydrogenase 2 family (mitochondrial)
8175683	1.55	microRNA 224
8138487	1.54	argininosuccinate synthetase pseudogene 11
8030557	1.48	activating transcription factor 5
8094778	1.48	ubiquitin carboxyl-terminal esterase L1 (ubiquitin thiolesterase)
7892609	1.48	ATP-binding cassette, sub-family F (GCN20), member 1
7956443	1.44	methionyl-tRNA synthetase
8070632	1.43	cystathionine-beta-synthase
8142270	1.42	neuronal cell adhesion molecule
8136200	1.41	carboxypeptidase A4
8042830	1.40	methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 2, methenyltetrahydrofolate cyclohydrolase
8176460	1.40	protein kinase, Y-linked
7976496	1.39	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3
8108217	1.38	transforming growth factor, beta-induced, 68kDa
8084064	1.38	ENSG00000181260
8138566	1.38	insulin-like growth factor 2 mRNA binding protein 3
8127201	1.38	collagen, type XXI, alpha 1
7956401	1.37	serine hydroxymethyltransferase 2 (mitochondrial)
8117422	1.33	histone cluster 1, H4l; histone cluster 1, H4k; histone cluster 4, H4; histone cluster 1, H4h; histone cluster 1, H4j; histone cluster 1, H4i; histone cluster 1, H4d; histone cluster 1, H4c; histone cluster 1, H4f; histone cluster 1, H4e; histone cluster 1, H4b; histone cluster 1, H4a; histone cluster 2, H4a; histone cluster 2, H4b
8158671	1.32	argininosuccinate synthetase 1
8002303	1.31	NAD(P)H dehydrogenase, quinone 1
8037835	1.28	solute carrier family 1 (neutral amino acid transporter), member 5
7914563	1.27	tyrosyl-tRNA synthetase
8084630	1.27	similar to hCG2041270
8109383	1.25	glutamate receptor, ionotropic, AMPA 1
8156783	1.23	collagen, type XV, alpha 1
7965979	1.21	aldehyde dehydrogenase 1 family, member L2
8154100	1.20	very low density lipoprotein receptor
7919749	1.20	RNA, 7SL, cytoplasmic 2; RNA, 7SL, cytoplasmic 1
8020955	1.19	molybdenum cofactor sulfurase
8060344	1.19	tribbles homolog 3 (Drosophila)
8095299	1.17	hypothetical LOC100271832; RNA, Ro-associated Y5 pseudogene 10; RNA, Ro-associated Y1; RNA, Ro-associated Y4 pseudogene 7; RNA, Ro-associated Y4 pseudogene 19; RNA, Ro-associated Y3; hypothetical LOC100132111; RNA, Ro-associated Y4

7982868	1.17	ChaC, cation transport regulator homolog 1 (E. coli)
8002347	1.16	alanyl-tRNA synthetase
7989985	1.15	integrin, alpha 11
7973530	1.14	phosphoenolpyruvate carboxykinase 2 (mitochondrial)
7969574	1.14	ENSG00000215209
8017599	1.14	platelet/endothelial cell adhesion molecule
8112045	1.13	endothelial cell-specific molecule 1
8154725	1.12	ENSG00000211510
7903619	1.10	seryl-tRNA synthetase
8162313	1.10	isoleucyl-tRNA synthetase
8028194	1.09	zinc finger protein 382
8022674	1.09	cadherin 2, type 1, N-cadherin (neuronal)
8036820	1.09	zinc finger protein 780A
7970949	1.08	mab-21-like 1 (C. elegans)
8136248	1.08	mesoderm specific transcript homolog (mouse)
8078600	1.08	transcription elongation factor A (SII), 1 pseudogene 2; transcription elongation factor A (SII), 1
8109938	1.07	RAN binding protein 17
7958174	1.07	thioredoxin reductase 1; hypothetical LOC100130902
8065359	1.07	CD93 molecule
8145889	1.06	eukaryotic translation initiation factor 4E binding protein 1
8042310	1.06	solute carrier family 1 (glutamate/neutral amino acid transporter), member 4
8091867	1.05	butyrylcholinesterase
8078529	1.05	SH3 and cysteine rich domain
8046380	1.05	integrin, alpha 6
8100393	1.04	kinase insert domain receptor (a type III receptor tyrosine kinase)
8099721	1.03	KIAA0746 protein
8108905	1.03	potassium channel tetramerisation domain containing 16
8036813	1.03	zinc finger protein 780B
8150818	1.02	transcription elongation factor A (SII), 1 pseudogene 2; transcription elongation factor A (SII), 1
8034099	1.02	microRNA 199a-1
7893128	1.00	asparaginyl-tRNA synthetase
8116494	1.00	zinc finger protein 62 homolog (mouse)
7956785	1.00	exportin, tRNA (nuclear export receptor for tRNAs); similar to Exportin-T (tRNA exportin) (Exportin(tRNA))
7920877	1.00	Rho/Rac guanine nucleotide exchange factor (GEF) 2

Supplementary Table S1: List of genes up-regulated in pediatric ASC.

Details about the 88 significantly up-regulated genes in pediatric versus adult ASC are presented with descending sorting according to log fold change.

Affymetrix reference	Log fold change	Description
8049532	-3.21	leucine rich repeat (in FLII) interacting protein 1
7976567	-2.95	bradykinin receptor B1
8049528	-2.68	leucine rich repeat (in FLII) interacting protein 1
8049540	-2.61	leucine rich repeat (in FLII) interacting protein 1
8117018	-2.49	RNA, U6 small nuclear 2; RNA, U6 small nuclear 1
8095680	-2.49	interleukin 8
8067040	-2.39	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2
8077441	-2.35	basic helix-loop-helix family, member e40
8152617	-2.34	hyaluronan synthase 2
8129573	-2.33	monooxygenase, DBH-like 1
7974335	-2.28	RNA, 5S ribosomal 9; RNA, 5S ribosomal 13; RNA, 5S ribosomal 12; RNA, 5S ribosomal 11; RNA, 5S ribosomal 10; RNA, 5S ribosomal 17; RNA, 5S ribosomal 16; RNA, 5S ribosomal 15; RNA, 5S ribosomal 14; RNA, 5S ribosomal 1; RNA, 5S ribosomal 2; RNA, 5S ribosomal 3; RNA, 5S ribosomal 4; RNA, 5S ribosomal 5; RNA, 5S ribosomal 6; RNA, 5S ribosomal 7; RNA, 5S ribosomal 8
8077499	-2.26	loss of heterozygosity, 3, chromosomal region 2, gene A
8114249	-2.24	chemokine (C-X-C motif) ligand 14
8049530	-2.20	leucine rich repeat (in FLII) interacting protein 1
8049542	-2.18	leucine rich repeat (in FLII) interacting protein 1
7954065	-2.10	G protein-coupled receptor, family C, group 5, member A
8043993	-2.07	AHPA9419
8101304	-2.02	RasGEF domain family, member 1B
8012906	-2.00	ENSG00000209646
8023220	-1.99	SMAD family member 7
8131803	-1.95	interleukin 6 (interferon, beta 2)
7968789	-1.95	chromosome 13 open reading frame 15
8122265	-1.94	tumor necrosis factor, alpha-induced protein 3
8068383	-1.92	chloride intracellular channel 6
7892850	-1.91	heterogeneous nuclear ribonucleoprotein C (C1/C2)
7898693	-1.88	alkaline phosphatase, liver/bone/kidney
7961580	-1.88	LIM domain only 3 (rhombotin-like 2)
8148304	-1.86	tribbles homolog 1 (Drosophila)
8107326	-1.84	small nucleolar RNA, H/ACA box 13
8100994	-1.84	chemokine (C-X-C motif) ligand 2
8083569	-1.83	TCDD-inducible poly(ADP-ribose) polymerase
8084878	-1.80	hypothetical LOC100271832; RNA, Ro-associated Y5 pseudogene 10; RNA, Ro-associated Y1; RNA, Ro-associated Y4 pseudogene 7; RNA, Ro-associated Y4 pseudogene 19; RNA, Ro-associated Y3; hypothetical LOC100132111; RNA, Ro-associated Y4
7914603	-1.80	ring finger protein 19B
8084880	-1.78	hairy and enhancer of split 1, (Drosophila)
8086330	-1.77	cysteine-serine-rich nuclear protein 1
8111443	-1.77	C1q and tumor necrosis factor related protein 3; alpha-methylacyl-CoA racemase
7973067	-1.76	nucleoside phosphorylase
8141016	-1.75	tissue factor pathway inhibitor 2
7978644	-1.74	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha
8041170	-1.74	small nucleolar RNA, C/D box 53
7952673	-1.74	FLJ45950 protein
8045835	-1.73	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 5 (GalNAc-T5)
7907249	-1.73	flavin containing monooxygenase 3
8070665	-1.72	salt-inducible kinase 1
8104607	-1.72	ENSG00000210072
8042211	-1.72	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 1; UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 2
7909730	-1.72	potassium channel, subfamily K, member 2
7965873	-1.70	insulin-like growth factor 1 (somatomedin C)
8041168	-1.69	small nucleolar RNA, C/D box 53
8114572	-1.67	heparin-binding EGF-like growth factor
7974337	-1.64	RNA, U6 small nuclear 2; RNA, U6 small nuclear 1
8095697	-1.62	chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)
7926037	-1.62	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3
7926545	-1.62	plexin domain containing 2
8111136	-1.60	family with sequence similarity 134, member B

7972217	-1.60	sprouty homolog 2 (Drosophila)
7955589	-1.58	nuclear receptor subfamily 4, group A, member 1
8131682	-1.58	ATP-binding cassette, sub-family B (MDR/TAP), member 5; small nuclear ribonucleoprotein 35kDa (U11/U12)
8037387	-1.57	RNA, 7SL, cytoplasmic 2; RNA, 7SL, cytoplasmic 1
7924450	-1.57	dual specificity phosphatase 10
7897801	-1.56	RNA, U5E small nuclear; RNA, U5A small nuclear; RNA, U5F small nuclear; RNA, U5B small nuclear 1; RNA, U5D small nuclear
8070557	-1.56	zinc finger protein 295
7917875	-1.55	coagulation factor III (thromboplastin, tissue factor)
8062461	-1.55	lipopolysaccharide binding protein
8057887	-1.54	serine/threonine kinase 17b
8151447	-1.54	interleukin 7
7965403	-1.54	lumican
7923547	-1.53	chitinase 3-like 1 (cartilage glycoprotein-39)
8144802	-1.53	platelet-derived growth factor receptor-like
8098103	-1.50	folliculin interacting protein 2
8040103	-1.49	inhibitor of DNA binding 2, dominant negative helix-loop-helix protein
7918768	-1.49	DENN/MADD domain containing 2C
7908459	-1.49	complement factor H
8055639	-1.48	ENSG00000176824
7900146	-1.48	zinc finger CCCH-type containing 12A
8068583	-1.47	potassium inwardly-rectifying channel, subfamily J, member 15
8143383	-1.47	ENSG00000208363
7907271	-1.47	flavin containing monooxygenase 2 (non-functional)
7922162	-1.46	solute carrier family 19 (thiamine transporter), member 2
7989883	-1.46	ENSG00000211307
7938702	-1.45	hypothetical protein DKFZp686O24166
7922610	-1.45	v-abl Abelson murine leukemia viral oncogene homolog 2 (arg, Abelson-related gene)
7957570	-1.44	plexin C1
8061013	-1.43	isthmin 1 homolog (zebrafish)
8152215	-1.43	Kruppel-like factor 10
7966052	-1.42	cryptochrome 1 (photolyase-like)
8140668	-1.41	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3A
8040080	-1.40	radical S-adenosyl methionine domain containing 2
8126093	-1.38	ENSG00000210733
8052231	-1.37	RNA, U6 small nuclear 2; RNA, U6 small nuclear 1
8123446	-1.37	SPARC related modular calcium binding 2
8172082	-1.37	ENSG00000210680
8007990	-1.37	hypothetical LOC100271832; RNA, Ro-associated Y5 pseudogene 10; RNA, Ro-associated Y1; RNA, Ro-associated Y4 pseudogene 7; RNA, Ro-associated Y4 pseudogene 19; RNA, Ro-associated Y3; hypothetical LOC100132111; RNA, Ro-associated Y4
7965206	-1.36	solute carrier family 6 (neutral amino acid transporter), member 15
7961514	-1.36	matrix Gla protein
8169061	-1.35	proteolipid protein 1
7965040	-1.35	pleckstrin homology-like domain, family A, member 1
8104022	-1.34	PDZ and LIM domain 3
7914342	-1.34	fatty acid binding protein 3, muscle and heart (mammary-derived growth inhibitor)
8138289	-1.34	ets variant 1
8149720	-1.34	early growth response 3
8065353	-1.33	thrombomodulin
8069553	-1.33	nuclear receptor interacting protein 1
8120043	-1.33	runt-related transcription factor 2
7936639	-1.33	small nucleolar RNA, H/ACA box 19
7902808	-1.32	heparan sulfate 2-O-sulfotransferase 1
7949264	-1.31	EH-domain containing 1
8157524	-1.31	toll-like receptor 4
8016841	-1.31	transmembrane protein 100
8025601	-1.31	intercellular adhesion molecule 1
8129763	-1.29	family with sequence similarity 54, member A
8178435	-1.29	immediate early response 3
8099685	-1.29	leucine-rich repeat LGI family, member 2
8146788	-1.29	ENSG00000211220

8123936	-1.27	neural precursor cell expressed, developmentally down-regulated 9
8055688	-1.27	Rho family GTPase 3
8166469	-1.26	spermidine/spermine N1-acetyltransferase 1
8101322	-1.26	MOP-1
8171837	-1.26	kelch-like 15 (Drosophila)
7893325	-1.26	heterogeneous nuclear ribonucleoprotein C (C1/C2)
8000649	-1.26	ENSG00000209903
8000690	-1.26	ENSG00000209903
8063382	-1.24	snail homolog 1 (Drosophila)
7905054	-1.24	RNA, U1F1 small nuclear; RNA, U1C2 small nuclear; RNA, U1G2 small nuclear; RNA, U1C1 small nuclear; RNA, U1G3 small nuclear; RNA, U1A3 small nuclear; RNA, U1G1 small nuclear; RNA, U1A small nuclear
7919160	-1.23	RNA, U1F1 small nuclear; RNA, U1C2 small nuclear; RNA, U1G2 small nuclear; RNA, U1C1 small nuclear; RNA, U1G3 small nuclear; RNA, U1A3 small nuclear; RNA, U1G1 small nuclear; RNA, U1A small nuclear
7919166	-1.23	RNA, U1F1 small nuclear; RNA, U1C2 small nuclear; RNA, U1G2 small nuclear; RNA, U1C1 small nuclear; RNA, U1G3 small nuclear; RNA, U1A3 small nuclear; RNA, U1G1 small nuclear; RNA, U1A small nuclear
7904361	-1.23	family with sequence similarity 46, member C
8022506	-1.23	RNA, U6 small nuclear 2; RNA, U6 small nuclear 1
8042144	-1.22	v-rel reticuloendotheliosis viral oncogene homolog (avian)
8078138	-1.22	ELL associated factor 1
8154692	-1.22	TEK tyrosine kinase, endothelial
7994265	-1.21	RNA, 5S ribosomal 9; RNA, 5S ribosomal 13; RNA, 5S ribosomal 12; RNA, 5S ribosomal 11; RNA, 5S ribosomal 10; RNA, 5S ribosomal 17; RNA, 5S ribosomal 16; RNA, 5S ribosomal 15; RNA, 5S ribosomal 14; RNA, 5S ribosomal 1; RNA, 5S ribosomal 2; RNA, 5S ribosomal 3; RNA, 5S ribosomal 4; RNA, 5S ribosomal 5; RNA, 5S ribosomal 6; RNA, 5S ribosomal 7; RNA, 5S ribosomal 8
8054722	-1.20	interleukin 1, beta
8068593	-1.20	v-ets erythroblastosis virus E26 oncogene homolog 2 (avian)
7912224	-1.20	solute carrier family 2 (facilitated glucose/fructose transporter), member 5
7979307	-1.20	discs, large (Drosophila) homolog-associated protein 5
8024485	-1.20	growth arrest and DNA-damage-inducible, beta
8175574	-1.19	SLIT and NTRK-like family, member 4
7933872	-1.19	early growth response 2
8088952	-1.19	ENSG00000211088
8180405	-1.19	ADP-ribosylation factor-like 4A
7936637	-1.19	small nucleolar RNA, H/ACA box 19
8021376	-1.19	neural precursor cell expressed, developmentally down-regulated 4-like
7936242	-1.19	inositol 1,4,5-triphosphate receptor interacting protein
8083616	-1.18	myeloid leukemia factor 1
8163002	-1.18	Kruppel-like factor 4 (gut)
7948088	-1.18	ENSG00000123443
7906863	-1.18	UDP-N-acteylglucosamine pyrophosphorylase 1
8075635	-1.17	ENSG00000183531
8029693	-1.17	FBJ murine osteosarcoma viral oncogene homolog B
7909225	-1.17	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 3
7933877	-1.17	jumonji domain containing 1C
8104568	-1.16	GALI1870
8078134	-1.16	ENSG00000209842
7942064	-1.16	galanin prepropeptide
7959102	-1.16	heat shock 22kDa protein 8
8002342	-1.16	ENSG00000209903
8086615	-1.16	leucine rich repeat containing 2
8131666	-1.16	integrin, beta 8
8124848	-1.15	immediate early response 3
8179704	-1.15	immediate early response 3
7908861	-1.14	ovarian cancer-related protein 1
7905220	-1.14	extracellular matrix protein 1
8119124	-1.13	peptidase inhibitor 16
8059996	-1.13	period homolog 2 (Drosophila)
8014664	-1.13	hypothetical LOC100271832; RNA, Ro-associated Y5 pseudogene 10; RNA, Ro-associated Y1; RNA, Ro-associated Y4 pseudogene 7; RNA, Ro-associated Y4 pseudogene 19; RNA, Ro-associated Y3; hypothetical LOC100132111; RNA, Ro-associated Y4

8019649	-1.13	hypothetical LOC100271832; RNA, Ro-associated Y5 pseudogene 10; RNA, Ro-associated Y1; RNA, Ro-associated Y4 pseudogene 7; RNA, Ro-associated Y4 pseudogene 19; RNA, Ro-associated Y3; hypothetical LOC100132111; RNA, Ro-associated Y4
7900365	-1.13	major facilitator superfamily domain containing 2
7983969	-1.13	cyclin B2
7897449	-1.13	splA/ryanodine receptor domain and SOCS box containing 1
8094169	-1.12	cytoplasmic polyadenylation element binding protein 2
8094056	-1.12	HtrA serine peptidase 3
8132318	-1.12	anillin, actin binding protein
8126750	-1.12	ectonucleotide pyrophosphatase/phosphodiesterase 5 (putative function)
8106403	-1.12	coagulation factor II (thrombin) receptor-like 1
7950933	-1.12	NADPH oxidase 4
8049512	-1.11	leucine rich repeat (in FLII) interacting protein 1
7962000	-1.11	parathyroid hormone-like hormone
7995258	-1.10	zinc finger protein 267
8178095	-1.09	complement component 2
8179331	-1.09	complement component 2
7933084	-1.09	nicotinamide phosphoribosyltransferase
7919815	-1.09	cathepsin K
8118324	-1.08	complement component 2
7980051	-1.08	chromosome 14 open reading frame 43
8014956	-1.08	nuclear receptor subfamily 1, group D, member 1
8037272	-1.08	pregnancy specific beta-1-glycoprotein 5
8160912	-1.07	chromosome 9 open reading frame 131
7944716	-1.07	ENSG00000211358
8165808	-1.07	Xg blood group
8142120	-1.06	nicotinamide phosphoribosyltransferase
8020179	-1.06	chromatin modifying protein 1B
7965838	-1.06	RNA, U6 small nuclear 2; RNA, U6 small nuclear 1
7999884	-1.05	ENSG00000209726
8088642	-1.05	leucine-rich repeats and immunoglobulin-like domains 1
8038890	-1.05	hyaluronan synthase 1
8072626	-1.05	TIMP metallopeptidase inhibitor 3
8140534	-1.05	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3C
7892676	-1.05	small nucleolar RNA, C/D box 18B; small nucleolar RNA, C/D box 18A; small nucleolar RNA, C/D box 18C
8129677	-1.05	serum/glucocorticoid regulated kinase 1
8108301	-1.05	kinesin family member 20A
7897460	-1.05	solute carrier family 25, member 33
8152703	-1.04	F-box protein 32
7926679	-1.04	KIAA1217
8088820	-1.03	RING1 and YY1 binding protein
7909708	-1.03	centromere protein F, 350/400ka (mitosin)
7965150	-1.03	RNA, 5S ribosomal 9; RNA, 5S ribosomal 13; RNA, 5S ribosomal 12; RNA, 5S ribosomal 11; RNA, 5S ribosomal 10; RNA, 5S ribosomal 17; RNA, 5S ribosomal 16; RNA, 5S ribosomal 15; RNA, 5S ribosomal 14; RNA, 5S ribosomal 1; RNA, 5S ribosomal 2; RNA, 5S ribosomal 3; RNA, 5S ribosomal 4; RNA, 5S ribosomal 5; RNA, 5S ribosomal 6; RNA, 5S ribosomal 7; RNA, 5S ribosomal 8
7904048	-1.02	ENSG00000209307
8113512	-1.02	erythrocyte membrane protein band 4.1 like 4A
7966878	-1.01	citron (rho-interacting, serine/threonine kinase 21)
8022902	-1.01	INO80 complex subunit C
7898057	-1.01	podoplanin
7926875	-1.01	hypothetical LOC729590; BMP and activin membrane-bound inhibitor homolog (Xenopus laevis)
8077490	-1.01	LIM and cysteine-rich domains 1
7974316	-1.01	FERM domain containing 6
7965812	-1.00	N-acetylglucosamine-1-phosphate transferase, alpha and beta subunits
7952249	-1.00	ubiquitin specific peptidase 2

Supplementary Table S2: List of genes down-regulated in pediatric ASC.

The details about 217 genes significantly down-regulated genes in pediatric versus adult ASCs are presented with descending sorting according to log fold change.

KEGG Pathways	Genes	P
Pathways with down-regulated genes in pediatric compared to adult ASCs		
NOD-like receptor signaling pathway	chemokine (C-X-C motif) ligand 1 chemokine (C-X-C motif) ligand 2 interleukin 1, beta interleukin 6 (interferon, beta 2) interleukin 8 nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha tumor necrosis factor, alpha-induced protein 3	Fold change: 6.2 p=0.04
Circadian rhythm	basic helix-loop-helix family, member e40 cryptochrome 1 (photolyase-like) nuclear receptor subfamily 1, group D, member 1 period homolog 2 (Drosophila)	Fold change: 17.0 p=0.05
Pathway with up-regulated genes in pediatric compared to adult ASCs		
Aminoacyl-tRNA biosynthesis	alanyl-tRNA synthetase asparaginyl-tRNA synthetase isoleucyl-tRNA synthetase methionyl-tRNA synthetase seryl-tRNA synthetase tyrosyl-tRNA synthetase tryptophanyl-tRNA synthetase	Fold change: 9.4 p=0.008

Supplementary Table S3: KEEG pathways of genes differentially expressed.

The details about the pathways involving genes differentially expressed between pediatric and adult ASCs are presented. Differentially expressed genes were annotated using KEGG_PATHWAY database from DAVID Bioinformatics Resources 6.7. Pathways were considered significantly enriched at 0.05 level. For each of them, the complete list of genes involved is provided; it has not been met with a situation where in a same pathway, both up and down regulated genes were found.

Genes	Foward primers	Reverse primers
AP2	AGTGAAAACTTTGATGATTATATG	CCATGCCAGCCACTTTCCT
CD31	GGAAAGCTGTCCCTGATGC	CATCTGGCCTTGCTGTCTAA
DLX5	TACCCAGCCAAAGCTTATGCCG	GCCATTCACCATTCTCACCTCG
eNOS	CATCACCAGGAAGAAGACCT	TCACTCGCTTCGCCATCA
FLT1	CAAATAAGCACACCACGCCC	CGCCTTACGGAAGCTCTCTT
GLUT1	ATACTCATGACCATCGCGCTAG	AAAGAAGGCCACAAAGCCAAAG
HGF	CAGAGGGACAAAGGAAAAGAA	GCAAGTGAATGGAAGTCCTTTA
hTERT	GGAGCAAGTTGCAAAGCATTG	TCCCACGACGTAGTCCATGTT
ICAM	CTGATGGGCAGTCAACAGCTA	CCTGGCAGCGTAGGGTAAG
IL-6	TCCACAAGCGCCTTCGGTCC	GCAGGGAAGGCAGCAGGCAA
IL-8	TGGCTCTCTTGGCAGCCTTCCT	TGGGGTGGAAAGGTTTGGAGTATGT
KDR	CGGTCAACAAAGTCGGGAGA	CAGTGCACCACAAAGACACG
LPL	GGTCGAAGCATTGGAATCCAG	TAGGGCATCTGAGAACGAGTC
MDM2	TGTTTGGCGTGCCAAGCTTCTC	CACAGATGTACCTGAGTCCGATG
MYC	CAAGTTCATAGGTGATTGCT	GCGAACACACAACGTC
NANOG	CAAAGGCAAACAACCCACTT	TCTGCTGGAGGCTGAGGTAT
OCT4-A	ACGACCATCTGCCGCTTTGAG	GCCTCTCACTCGGTTCTCGAT
Osteocalcine	CACTCCTCGCCCTATTGGC	CCCTCCTGCTTGGACACAAAG
Osterix	ATGGCGTCCTCCCTGCTTGAG	AGGGGTGTGTCATGTCCAGAGAGG
p16	CACCGGGTCGGGTGAGAGT	CCCAACGCACCGAATAGTTAC
p16-FAM	AGGCCGATCCAGGTCATGATGATGG	
p21	CTGGAGACTCTCAGGGTCGAAAA	TGTAGAGCGGGCCTTTGAGG
p53	TAACAGTTCCTGCATGGGGGGC	AGGACAGGCACAAACACGCACC
PPARγ2	GATACACTGTCTGCAAACATATCAC	CCACGGAGCTGATCCCAA
PPIA	GCCGAGGAAAACCGTGTACTAT	TCTTTGGGACCTTGTCTGCAA
PTHR1	ACATCTGCGTCCACATCAGG	CCGTTCACGAGTCTCATTGGTG
PUM1	AGTGGGGGACTAGGCGTTAG	GTTTTCATCACTGTCTGCATCC
retinoblastoma 1	CAGAAGGTCTGCCAACACCAAC	TTGAGCACACGGTCGCTGTTAC
RunX2	ATTCCTGTAGATCCGAGCACC	GCTCACGTCGCTCATTTTGC
SNAIL 1	CCTTCGTCCTTCTCCTC	TGACATCTGAGTGGGTCTGG
SOX2	GCTACAGCATGATGCAGGACCA	TCTGCGAGCTGGTCATGGAGTT
TGFbeta2	CTGTGCTGGAGCATGCCCGT	TGGGACACGCAGCAAGGAGA
TNFalpha	CAGGCGCCACCACGCTCTTC	CTGGGGAACTCTTCCCTCTGGGG
VEGF	CTCTACCTCCACCATGCCAAG	AGACATCCATGAACTTCACCACTTC
vWF	GATGGAGTCCAGCACCAGTT	GCTACTTCACACAGGCCACA
YWHAZ	AGCAGGCTGAGCGATATGAT	TCTCAGCACCTTCCGTCTTT

Supplementary Table S4: Details of the primer sequences used for qPCR.

Primers were designed using Primer Express software (Applied Biosystems,) and validated by testing PCR efficiency using standard curves (85%≤ efficiency ≤115%), allowing us to use the ΔΔCt method for absolute quantification. PCR product specificity was evaluated by generating a dissociation curve following the manufacturer's recommendation.

MONSARRAT Paul

Cellules souches, médecine régénérative et régénération parodontale

Directeur de thèse

Dr Valérie Planat-Bénard et Pr Philippe Kémoun

Université Toulouse III, le 25 janvier 2016

RESUME

La première partie de ce travail introduit un nouveau concept d'analyse des enregistrements des essais cliniques et de la dynamique de leur évolution, aussi bien thématique que temporelle. Ce concept a été appliqué à la médecine régénérative, démontrant l'absence de corrélation entre la source de cellules souches et le champ d'application. Les pathologies odonto-stomatologiques sont très peu concernées par les essais cliniques en thérapie cellulaire par cellules souches. Pourtant les parodontites, pathologies immuno-infectieuses responsables de la destruction du tissu de soutien des dents, constituent un enjeu majeur de santé publique. Bien que les auteurs s'accordent sur la responsabilité de l'écologie immunitaire et microbienne dans la physiopathologie de la maladie, les raisons de la dysbiose, de la susceptibilité individuelle sont encore mal connues. La greffe de cellules stromales mésenchymateuses (CSM) permettrait le retour à l'homéostasie en favorisant l'activation des CSM endogènes.

La deuxième partie de ce travail démontre que les parodontites ont été potentiellement associées avec 57 pathologies systémiques ; le registre des essais cliniques de l'Organisation Mondiale de la Santé ayant été analysé. L'efficacité et la sureté de l'utilisation des CSM pour la régénération parodontale dans des modèles animaux ont été également démontrées. Pourtant, les modèles utilisés souffraient de problèmes méthodologiques dont la faible représentativité physiopathologique des lésions parodontales générées. Cette deuxième partie apporte donc des données quant à l'efficacité des ASC (CSM du tissu adipeux) pour améliorer de manière quantitative et qualitative la régénération des tissus de soutien parodontaux dans un modèle murin où les lésions parodontales ont été générées par l'administration répétée de bactéries parodonto-pathogènes. Il s'agit donc d'un modèle dont la physiopathologie est plus proche de celle retrouvée chez l'Homme. Enfin, la deuxième partie démontre un effet antibactérien à large spectre des ASC dont l'effet est à la fois direct (effet macrophage-like) et indirect (via la sécrétion de facteurs antibactériens).

Mots clefs

Cellules stromales mésenchymateuses, Cellules souches, Tissu adipeux, Data mining, Big Data, Essais cliniques, Bactéries, Parodontite.

Discipline : Physiopathologie

Unité de recherche : CNRS 5273 ; UMR STROMALab ; Université de Toulouse UPS ; INSERM U1031 ; EFS Pyrénées – Méditerranée ; Toulouse, France.

MONSARRAT Paul

Stem cells, regenerative medicine and periodontal regeneration

The first part of this work introduces a new concept of analysis of clinical trial records and the dynamics of their evolution, both thematic and temporal. This concept has been applied to regenerative medicine, showing the lack of correlation between the source of stem cells and the fields of application. The stomatognathic diseases are few involved in clinical trials for stem cells therapy. Yet periodontitis, immuno-infectious diseases responsible for the destruction of the tooth supporting tissues, are a major public health issue. While the authors agree on the responsibility of the immune and microbial ecology in the pathophysiology of the disease, the reasons for dysbiosis, individual susceptibilities, are still unclear. Graft of mesenchymal stromal cells (MSCs) would return to homeostasis by promoting the activation of endogenous MSCs.

The second part of this work shows that periodontitis were potentially associated with 57 systemic diseases; the clinical trials registry of the World Health Organization have been analyzed. The efficacy and safety of the use of MSCs for periodontal regeneration in animal models have also been demonstrated. Yet the models suffered from methodological problems, periodontal lesions are few representative of the pathophysiology. This second part thus provides data on the effectiveness of ASC (CSM from adipose tissue) to improve quantitative and qualitative regeneration of periodontal supporting tissues in a mouse model where periodontal lesions were generated by repeated administration of parodonto-pathogenic bacteria. It is therefore a model whose pathophysiology is closer to that found in humans. Finally, the second part demonstrates broad antibacterial spectrum of ASC whose effect is both direct (macrophage-like effect) and indirect (via the secretion of antibacterial factors).