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Review

Stem cells in dentistry – Part II: Clinical applications

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Abstract

New technologies that facilitate solid alveolar ridge augmentation are receiving considerable attention in the field of prosthodontics because of the growing requirement for esthetic and functional reconstruction by dental implant treatments. Recently, several studies have demonstrated potential advantages for stem-cell-based therapies in regenerative treatments. Mesenchymal stem/stromal cells (MSCs) are now an excellent candidate for tissue replacement therapies, and tissue engineering approaches and chair-side cellular grafting approaches using autologous MSCs represent the clinical state of the art for stem-cell-based alveolar bone regeneration. Basic studies have revealed that crosstalk between implanted donor cells and recipient immune cells plays a key role in determining clinical success that may involve the recently observed immunomodulatory properties of MSCs. Part II of this review first overviews progress in regenerative dentistry to consider the implications of the stem cell technology in dentistry and then highlights cutting-edge stem-cell-based alveolar bone regenerative therapies. Factors that affect stem-cell-based bone regeneration as related to the local immune response are then discussed. Additionally, pre-clinical stem cell studies for the regeneration of teeth and other oral organs as well as possible applications of MSC-based immunotherapy in dentistry are outlined. Finally, the marketing of stem cell technology in dental stem cell banks with a view toward future regenerative therapies is introduced.

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Keywords: Bone regeneration; Immunotherapy; Implant dentistry; Stem cells; Tissue engineering; Tooth regeneration

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1. Introduction

Stem cells play vital roles in the repair of every organ and tissue through their capacity for self-renewal and differentiation. Part I of this review outlines various stem cell sources in oral and maxillofacial tissues with regard to clinical availability and applications in dentistry [1]. The oral area is a rich and unique source of stem cells, and it is therefore important for dental clinicians and researchers to further characterize these cells to develop new and effective strategies for dental applications.

The focus of stem cell research in dentistry is the regeneration of missing oral tissues. In particular, the restoration of alveolar ridge height is a major concern to prosthodontists because bone defects that arise after tooth loss usually result in further horizontal and vertical bone loss [2], which limits the effectiveness of dental implants and other prosthodontic treatments [3]. Therefore, stem-cell-based regenerative technology is considered to represent a new frontier in prosthodontic medicine [4].

In tissue engineering, the important elements for tissue regeneration are not only stem cells but also biomaterial scaffolds (cell-instructive templates) and growth and differentiation factors (biologically active molecules) [5]. In this regard, conventional regenerative dentistry has already developed scaffold and growth factor technologies (see Section 2). To achieve efficient oral tissue regeneration, however, it is necessary to combine the existing material-based technologies and anticipated stem cell-based technologies. Therefore, a solid knowledge of biomaterials and growth factors is also necessary to create stem-cell-based strategies for oral tissue engineering.

Stem cell technology for regenerative therapies is already available, as mesenchymal stem/stromal cells (MSCs) already have been introduced in the clinic for alveolar bone augmentation (see Section 4), relatively little is known about their *in vivo* biology. However, the translational utility of stem-cell-based technologies is still uncertain because the effectiveness of such approaches when compared with already-established regenerative techniques has not yet been properly evaluated, especially when considering their high cost and labor required. In addition, it is important to not overestimate the regenerative properties of stem cells, because most studies to

date have not considered the negative effects of the host immune system on transplanted cells. Therefore, factors that affect stem-cell-based therapies in the donor and recipient need to be addressed before therapeutic effects can be realized (see Section 5).

In the field of prosthodontics, especially in the clinic, material-based reconstruction without major surgical procedures was the main approach to treatment; however, emerging stem cell technologies and the requirements of alveolar ridge augmentation associated with implant dentistry have expanded the clinical concept to include stem-cell-based regeneration (see Section 3.1). Stem cell technologies have even permitted dental scientists to imagine the development of bioengineered teeth to replace the patient's missing teeth (see Sections 3.2 and 6.1). Furthermore, "dental stem cell banking" is already on the market for possible future use in regenerative therapies (see Section 7). Thus, clinicians as well as researchers in the prosthodontic field should understand basic aspects of stem cells and the implications of stem cell technologies in the future of dentistry.

In this review, we first overview conventional material-based regenerative dentistry to consider the necessity of stem cells for further advances, and we then describe the current status of stem-cell-based therapies in dentistry. Challenging issues regarding the factors that affect stem-cell-based bone regeneration and that need to be addressed for the clinical success of stem-cell-based strategies are then addressed. Additionally, pre-clinical stem cell studies for complex oral tissue/organ engineering and cell-based immunotherapy using MSCs are outlined. Finally, the marketing of stem cell technology in dental stem cell banks is introduced.

2. Progress in regenerative therapy in dentistry

In addition to the oro-maxillofacial reconstruction of tissues lost to trauma or cancer [6,7], the concept of regenerative dentistry has especially applied in the fields of periodontology and implantology [8,9] because periodontal disease is a common cause of alveolar bone and tooth loss that limits the ability of dental implants to restore the periodontal anatomy or missing teeth. This section discusses the progress in regenerative therapies related to periodontal tissue and alveolar bone (Fig. 1).

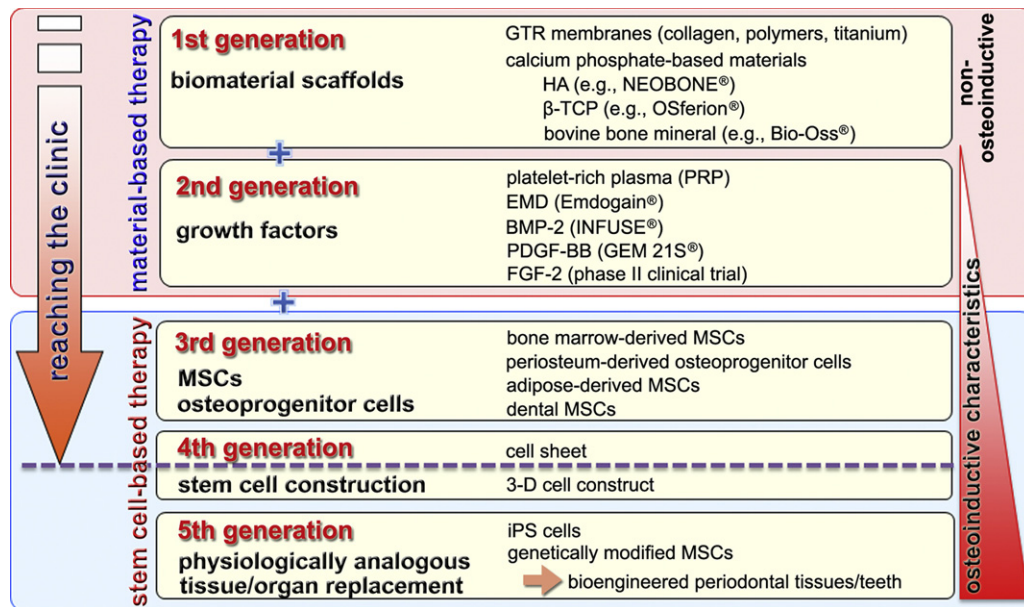


Fig. 1. Progress in regenerative periodontal/bone therapies. Regenerative periodontal/bone therapies are broadly categorized as material-based therapies (first-generation biomaterial scaffold-based approach and second-generation growth-factor-based approach) and stem-cell-based therapies (third-generation MSC/osteoprogenitor cell-based approach, fourth-generation stem-cell construction-based approach, and fifth-generation physiologically analogous tissue/organ-replacement approach). Technologies from the first to the fourth generation have already reached the clinic. See details in Section 2.

Regenerative periodontal/bone therapy was originally based on the use of scaffolds. In this first generation of this approach, osteoconductive membranes and bone graft materials were used as a framework for cells migrate into the periodontal tissue to allow it to regenerate at its normal healing rate. The second generation utilized osteoinductive materials, such as growth factors, to stimulate periodontal tissues to grow at an increased rate. Treatment protocols based on these concepts have already widely infiltrated general dental practice because they utilize only non-viable materials during the surgical process and are therefore easily applied.

In contrast, MSC-based regenerative therapies have been established as a third-generation regenerative periodontal/bone therapy mainly in clinical research facilities such as university hospitals. Cell construction technologies [10–12], such as cell sheets, have recently been introduced to regenerative dentistry as a fourth-generation approach, and clinical trials are now under way. Future fifth-generation approaches are expected to use oral tissue-derived induced pluripotent stem (iPS) cells [13–15] and genetically modified stem cells to create more physiologically analogous replacement tissue/organs, such as bioengineered periodontal tissues/teeth.

Combinations of these approaches, *e.g.*, simultaneous application of scaffolds, growth factors, and stem cells, are expected to increase the efficacy of regenerative therapies, based on the traditional tissue engineering concept [5]. However, the extent of clinical acceptance may vary greatly between material-based therapies (*i.e.*, the first- and second-generation approaches) and stem cell-based therapies (>third-generation approaches), which are practically difficult to implement in general dental clinics. Thus, regenerative approaches in future dental treatments should be discussed in two categories according to whether or not they utilize stem

cells. The following sections overview each treatment approach to discuss the needs of stem-cell-based therapies in addition to material-based therapies for clinical success.

2.1. Scaffold-based tissue regeneration

The basic concept underlying conventional periodontal regenerative therapy is first to remove the source of infection and then to provide a space into which neighboring cells can grow [16]. To this end, various types of bone grafting materials have been applied to periodontal defects. The most documented material-based regenerative technique for periodontal regeneration therapy is guided tissue regeneration (GTR) [17,18], in which biocompatible barrier membranes, such as resorbable collagen (BioMend[®]: Calcitek, Colla-Tec Inc., USA) and poly lactic-co-glycolic acid (PLGA: GC membrane, GC Corporation, Japan) membranes or non-resorbable expanded polytetrafluoroethylene (ePTFE: GORE-TEX Regenerative Membrane[®], W.L. Gore & Associates, Inc., USA) and titanium (Jeil Ti mesh, ProSeed, Japan) membranes, are surgically implanted to cover and protect the bone defect. In this procedure, connective tissue and bone regeneration then occur within the bone defect, which is protected by the barrier from rapid migration of epithelial tissues into the wound [19].

The PLGA and ePTFE polymers and commercially pure titanium membranes are bioinert materials that do not stimulate bone formation and do not directly bond to bone [20]. Therefore, alveolar bone augmentation/preservation techniques, such as guided bone regeneration (GBR) and socket preservation, require the use of bioactive materials, such as calcium phosphate (CaP)- and collagen-based grafts, to stimulate bone tissue formation and thus provide direct bonding with bone. Representative CaP-based biomaterials

include hydroxyapatite (HA: NEOBONE[®], Covalent Materials, Japan) [21], tricalcium phosphate (β -TCP: OSferion[®], Olympus, Japan), biphasic calcium phosphate (HA + β -TCP: Triosite[™], Zimmer, France) and bovine bone mineral (BBM: Bio-Oss[®], Geistlich Biomaterials, Switzerland).

It should be noted that CaP-based biomaterials are bioactive and osteoconductive, but they are not osteoinductive because they do not induce the formation of *de novo* bone in non-osseous sites [22]. Clinically, osteoinduction by bone grafting substitutes is especially important when applying titanium dental implants to permit accelerated bone formation and enhanced osseointegration of the implants with bone, thereby minimizing implant loosening that could lead to implant failure. Therefore, osteoinductive CaP-based scaffolds have been engineered through the incorporation of osteogenic bioactive factors (see Section 2.2 for the growth factors in the regenerative dentistry) and have been shown to promote bone formation [8,23,24].

As an alternative, bone graft/scaffold engineering using fibrous silk protein (fibroin) biomaterials from silkworms and spiders has received increasing interest [25] because of the controllable porosity, surface roughness and stiffness of these materials in the 3D scaffold fabrication process [26], and because they can be functionalized by chemical coupling of bioactive molecules or covalent conjugation of osteogenic growth factors [27]. Currently, the efficacy of silk-based biomaterials for bone regeneration has been investigated in pre-clinical studies, but these materials have not yet reached human application.

It is often beneficial for scaffolds to mimic the natural extracellular matrix (ECM) because ECM components specifically modulate MSC adhesion, migration, proliferation and osteogenic differentiation [28]. However, it is difficult to use animal-derived ECM clinically because of safety issues; synthetic peptide analogues of ECM components [29,30] or bioactive small molecules [31] may thus represent promising alternatives. When the fabrication cost is taken into account, cell-derived decellularized extracellular matrices may also present a promising approach to obtain ECM-based biomimetic materials [32].

Regardless of the material type, the challenge in scaffold-based tissue regeneration strategies is to determine the appropriate scaffold properties (*e.g.*, porosity, surface geometry and mechanical strength) to support the cell activity necessary to promote bone regrowth by the host cells. In addition, appropriate carrier properties of the scaffolds should be determined to provide controlled release of osteogenic bioactive factors.

2.2. Tissue regeneration based on growth factor delivery

Growth factor delivery has increased the options for combinatorial approaches with scaffold-based tissue regeneration. It is well known that the sequential bone development cascade is organized by a variety of cells and trophic/growth factors [33,34]. The tissue regeneration process can be partially considered as a recapitulation of the normal development

process; therefore, it is reasonable to use trophic/growth factors to recruit stem cells to tissue defects and stimulate them to achieve regeneration.

One representative therapy that uses growth factor delivery to achieve periodontal regeneration is the application of platelet-rich plasma (PRP), which consists of autologous platelets concentrated in a small volume of plasma. PRP contains several different growth factors and matrix elements [35] that may be used to regenerate periodontal defects. Currently, there is great interest concerning the use of PRP in combination with bone grafts or autologous stem cells [36] to obtain predictable periodontal regeneration. A recent systematic review showed that PRP was beneficial in the treatment of periodontal intrabony defects when used with graft materials but not with GTR [37]. Another report suggested that PRP may not provide significant benefits when compared with the use of β -TCP alone in the treatment of three-walled intrabony defects [38]. A split-mouth clinical trial that evaluated the effects of PRP on sinus lifting showed that bone augmentation upon histological observation was significantly increased in sites treated with BBM (Bio-Oss[®]) plus PRP when compared with BBM alone, although clinical assessments (computed tomography (CT) densitometry and the height of the augmented bone) showed no significant differences between these treatment modalities [39]. The inconclusive results of clinical trials of PRP may in part be derived from variations in platelet count and growth factor components among different PRP preparation techniques [35]. Nonetheless, at this time, there is no human study that strongly supports the use of PRP to treat severe alveolar bone loss, such as in sinus lifting procedures [40].

A commercially available enamel matrix derivative (EMD) product (Emdogain[®], Biora AB, Sweden) has also been widely used in periodontal regeneration [41,42]. EMD is extracted from developing porcine tooth buds and has been reported to be composed primarily of amelogenin. Despite its encouraging clinical outcomes, the mechanisms underlying the effects of Emdogain[®] on periodontal regeneration are not yet clear. Several studies suggest that EMD stimulates periodontal fibroblast proliferation/growth and inhibits epithelial cell proliferation/growth, which may thus lead to periodontal tissue regeneration [41–43]. However, a recent systematic review indicated a lack of additional benefit of a combined therapy of GTR and EMD in infrabony or furcation defects when compared with GTR therapy alone [44]. Because PRP and EMD are composed of various different proteins, it is important to identify their definitive active ingredients to obtain optimal and predictable clinical outcomes.

Recently, several recombinant growth factors have been introduced for periodontal/bone regenerative therapy [45], including bone morphogenetic protein (BMP)-2 [46], platelet-derived growth factor (PDGF)-BB [47,48] and fibroblast growth factor (FGF)-2 [49,50]. BMP was originally characterized by its ability to induce bone formation [51,52]. Currently, BMPs are also known to play important roles in embryonic patterning and early skeletal formation [53]. Among the members of the BMP family, BMP-2 is famous for its strong ability to induce bone and cartilage formation [51–54].

Although BMP-2 is dispensable for bone formation [55], it is considered to activate bone-forming cells in the regenerative niche, such as stem cells and osteogenic progenitor cells, to induce the formation of new bone. Clinically, INFUSE[®] (Bone Graft, Medtronic, USA), which consists of recombinant human BMP-2 and an absorbable collagen sponge carrier, has been reported to dramatically induce bone formation in sinus augmentation and alveolar ridge augmentation [45,46,50].

Another commercially available growth-factor-based graft material for dental surgical procedures is GEM 21S[®] (Osteohealth, USA), which combines recombinant human PDGF-BB and β -TCP. PDGF, which is an important factor in PRP, is known to induce the formation and growth of blood vessels [56,57]. In dental applications, PDGF treatment enhances the proliferation of gingival and periodontal ligament (PDL) fibroblasts as well as cementum formation around teeth with periodontal defects [47]. Current available evidence supports the use of rhPDGF with a β -TCP graft to promote periodontal and peri-implant bone regeneration [48]. A recent randomized, controlled clinical trial confirmed that the local application of PDGF-BB in a β -TCP scaffold promoted long-term stable clinical improvements for patients afflicted by localized periodontal defects [58].

FGF-2 is another promising candidate for growth factor delivery [49,50,59], as it has a wide variety of biological functions in tissue regeneration, such as inducing angiogenesis (the formation of new blood vessels) and stem cell proliferation. A phase II clinical trial has already been completed [60], and the efficacy of gel-like formulated FGF-2 for periodontal regeneration has been confirmed [49]. Future therapies may thus use various combinations of growth factors to promote optimal periodontal tissue regeneration.

The conditioned medium from MSC cultures has also recently been reported to enhance bone formation in an experimental calvarial defect in rats [61]. The conditioned medium contains insulin-like growth factor (IGF)-1 and vascular endothelial growth factor (VEGF), but not FGF-2, PDGF-BB or BMP-2 [61]. Notably, the conditioned medium from cultured MSCs had stronger bone formation activity than the MSCs themselves. However, the detailed composition and underlying bone formation mechanism for the conditioned medium have not yet been reported. The active components and the optimal concentration of the conditioned medium should be determined prior to clinical application.

3. Requirements of stem cells in regenerative therapy

3.1. Alveolar bone augmentation

Clinical outcomes of material-based treatments indicate that partial periodontal tissue/bone loss (infrabony or furcation defects) can be treated using bioactive materials in a local environment that is suitable for natural healing, *i.e.*, one that enhances the capacity of local resident stem cells and their niche to regenerate the tissue. If partial periodontal tissue regeneration is desired in a patient, a material/growth-factor-based therapy should be the first choice because stem-cell-based

therapies carry the drawbacks of high cost and labor. However, variability in clinical outcomes has been reported for material/growth-factor-based regenerative therapies, which can be generally considered to have unpredictable results [62,63]. Therefore, improved bioactivity of materials for tissue regeneration and careful case selection and treatment planning are necessary to optimize the treatment outcomes.

It is clinically evident; however, that bone augmentation of the severely atrophic alveolar ridge, particularly vertical bone augmentation during GBR or sinus-lifting procedures, cannot be easily accomplished through material/growth-factor-based approaches alone because conventional bone grafting materials are not osteoinductive. Therefore, unavoidable resorption is induced by activated osteoclasts as an immune response against the transplants. Even if osteoinductive growth factors are applied with the scaffolds, their effect may be insufficient for the host cells to migrate into the large defect space.

Thus, autologous cancellous bone has been conventionally used for large bone defects because it possesses osteogenic, osteoconductive and osteoinductive properties provide by its appropriate cellular content [64]. However, autologous bone grafts exhibit high variability in their osteogenic potential among harvest sites and individuals [65], which can result in a less-than-desirable clinical outcome. In addition, difficulty in harvesting, limited intraoral supply, and associated donor site morbidity observed for autologous grafts have encouraged the development of stem-cell-based tissue engineering therapy as an alternative method. In this approach, the transplantation of stem cells into a large defect site would enable the grafted cells to respond to signaling molecules in the periodontal/osteogenic microenvironment to regenerate the tissue.

The recent increase in the demand for dental implants has generated a need for robust bone augmentation in the atrophic alveolar ridge and the maxillary sinus. The Academy of Osseointegration stated in its 2010 Silver Anniversary Summit [66] that the continued improvement of the dental implant success rate will require stem cell-based technologies, as osteogenic stem cells in an implant osteotomy site could provide the necessary factors to form superior bone that could contribute to enhanced long-term success of the implant treatment. Such an approach would decrease the need for a GTR membrane and could be used as a single product without requiring other adjuncts. Stem cell therapy is also potentially important for patients with compromised vascular supply and impaired wound healing because it may be able to improve vascularity to facilitate hard tissue augmentation at local sites [66]. Therefore, stem cells seem to present a promising strategy to achieve the regeneration of large alveolar bone defects, particularly to provide stable and accelerated bone formation as well as enhanced osseointegration in dental implant treatments.

3.2. Tooth regeneration

Tooth regeneration has long been desired as the ultimate dental treatment because humans only have two sets of teeth: the deciduous and permanent teeth. Although predictable clinical effectiveness has been recognized for

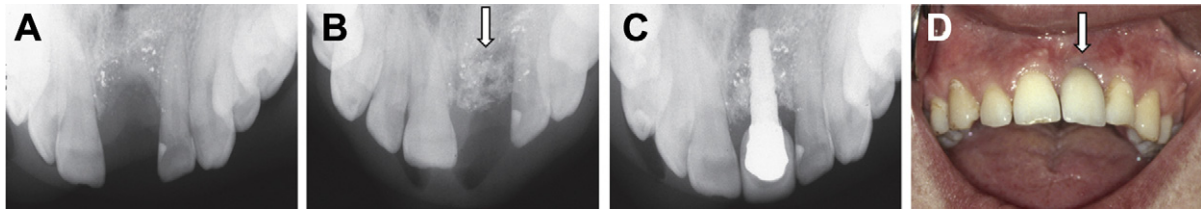


Fig. 2. A clinical case of stem-cell-based alveolar bone regeneration for the insertion of a dental implant. A 20-year-old male patient presented with a missing maxillary left central incisor that had been lost in an accident. (A) Radiograph showing the significant alveolar bone defect that resulted from the loss of the tooth. (B) Radiograph showing the augmentation of the alveolar bone (arrow) by stem-cell-based therapy. *Ex vivo*-expanded bone marrow mesenchymal stem/stromal cells from the iliac crest were applied to the defect in a hydroxyapatite scaffold. (C) Radiograph showing the dental implant insertion four months after stem-cell treatment. (D) Labial view of the implant restoration (arrow). These images are from the 2007 study by Meijer et al. in *PLoS Medicine* [138]. The figure was reproduced under the open-access license policy of the journal.

tooth replacement using titanium dental implants [67–70], dental implants do not function identically to natural teeth because they integrate directly into the bone without an intervening PDL through a process known as osseointegration [71]. In natural teeth, the PDL serves a sensory function and makes it possible to absorb and distribute loads produced during mastication and other types of tooth contact. In addition, the PDL plays a critical role in tooth movement and in maintaining homeostasis of the PDL and alveolar bone.

Clinically, a drawback of implant treatments is that the implanted material cannot adapt itself to changes in the surrounding tissues during the growth or aging of the patient [72–75]. Indeed, we occasionally encounter clinical cases in which the incisal edge of the implant-supported superstructure and the adjacent teeth become unevenly aligned years after the implantation. In addition, we occasionally encounter clinical cases that show unexpected recession of the facial mucosal margin at the implant site [76,77] or fracture of the implant or its superstructure as well as bone loss in patients with bruxism [78]. These problems appear to be caused at least in part by the lack of soft tissue homeostasis and cushioning normally provided by the PDL. Furthermore, recent reports have raised the possibility of metal sensitivity after exposure to titanium in some patients under certain circumstances [79,80]. Therefore, implant treatments require improvement in several aspects, such as tooth movement, tissue homeostasis, shock absorption, and anti-allergic biocompatibility. These requirements have gradually engendered the necessity of stem-cell-based tooth regeneration to ameliorate the deficiencies of titanium dental implants.

4. Current status of stem-cell-based therapy

The clinical effectiveness of stem cell therapies has mainly been evaluated in alveolar ridge augmentation for the insertion of dental implants (Fig. 2). Currently, clinical approaches to stem-cell-based bone augmentation are divided broadly into two categories: a tissue engineering approach and a chair-side cellular grafting approach (Fig. 3). In both approaches, bone marrow-derived MSCs (BMSCs) from the iliac crest are the most commonly used stem cells because they are the most well characterized stem cells among clinically available stem cells and have been shown to possess superior osteogenic ability. In addition, periosteum-derived stem/osteoprogenitor cells

[81–87], adipose tissue-derived MSCs [88,89] and dental tissue-derived MSCs [90] have been applied to engineer bone for orofacial bone regeneration (the merits of each type of stem cell for the regeneration of alveolar bone are described in detail in Part I of this review [1]).

4.1. Tissue engineering approach

Conventional and long-established stem-cell-based regenerative strategies have used cell culture techniques to increase the number of cells *in vitro* for later implantation to achieve bone tissue engineering (Table 1). In 2003, Schmelzeisen et al. [81] first showed the feasibility of using a tissue-engineered bony graft formed by periosteum-derived stem/osteoprogenitor cells for augmentation in the posterior maxilla prior to implant insertion. The following year, the same group demonstrated from clinical results in 27 patients that lamellar bone formed within 3 months after transplantation to provide a reliable basis for the insertion of dental implants [82]. Recently, Nagata et al. [87] reported using histomorphometric and CT analyses that the application of cultured periosteal cells with particulate bone and PRP as an autologous glue-like graft material induced bone remodeling, thereby enhancing osseointegration and consequently reducing postoperative waiting time after dental implant placement. It was suggested that the grafted cell-based material may serve as a source of stem cells, osteoprogenitor cells and angiogenic cells to accelerate the regeneration of functional bone with metabolic activity by supplying cells and growth factors necessary to activate bone formation and resorption.

In 2004, Ueda and his colleagues demonstrated successful alveolar bone tissue engineering with simultaneous implant placement through the application of an injectable gel-like mixture of BMSCs and PRP [91]. The same group subsequently reported successful tissue engineering in cases of periodontal bone loss [36], alveolar cleft osteoplasty [92], and maxillary sinus floor elevation [93,94]. The effectiveness of BMSCs for orofacial bone regeneration and implant placement has also been demonstrated when the cells are applied with HA particles [95], biphasic HA/ β -TCP [96], a gelatin sponge [97], BBM (Bio-Oss[®]), HA/TCP and recombinant PDGF [98], and frozen autologous cancellous bone [99]. In addition to BMSCs, adipose tissue-derived MSCs have also been shown to be useful for orofacial bone regeneration and implant placement [88,89].

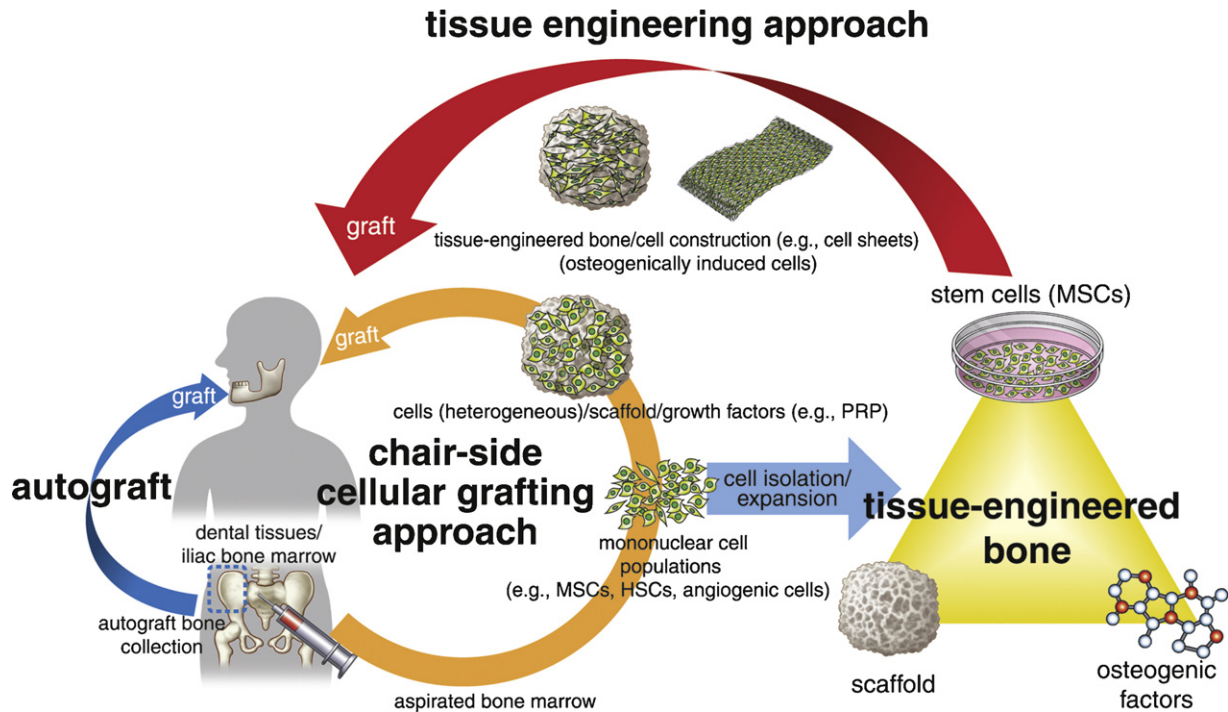


Fig. 3. Schematic diagram illustrating the current clinical approaches to stem-cell-based bone augmentation. The chair-side cellular grafting approach (orange arrow) uses patient-derived freshly processed bone marrow (mononuclear cell population), which contains mesenchymal stem/stromal cells (MSCs), hematopoietic stem cells (HSCs), and angiogenic cells, mixed with a scaffold and growth factors, such as platelet-rich plasma (PRP), as a grafting material. The tissue engineering approach (red arrow) uses MSCs, which are isolated from aspirated bone marrow and expanded *in vitro*. The MSCs are further cultured with osteogenic factors and a scaffold to generate an osteogenic construct (tissue-engineered bone) or cell sheets as a grafting material. Conventional autograft bone augmentation (blue arrow) uses autologous bone collected from the ilium or mandible.

Dental pulp-derived MSCs also demonstrated the capacity to completely restore human mandible bone defects when they used with a collagen sponge scaffold [90].

Although these reports suggest that stem-cell-based tissue engineering is beneficial, general critiques of cell therapy approaches have included the lack of characterization of the cellular component of the graft and the lack of reproducible cell isolation and expansion protocols that can predictably yield consistent cell populations. Indeed, Meijer et al. [95] reported substantial interpatient variability in clinical bone formation when using a stem-cell-based tissue engineering therapy. To overcome these issues, Kaigler et al. [100] prepared a standardized MSC population that was enriched in CD90- and CD14-positive cells using an automated cell processing unit, and reported a randomized, controlled feasibility trial for the regeneration of craniofacial bone. The stem-cell-based therapy accelerated alveolar bone regeneration when compared with GBR therapy. Zizelmann et al. [84] evaluated the resorption rate of tissue-engineered bone grafts and autologous cancellous bone in the maxillary sinus 3 months after operation, and suggested that tissue-engineered grafts containing cultured periosteum-derived osteoblasts (resorption rate of 90%) were less reliable than autologous bone grafts (resorption rate of 25%) for sinus augmentation. Further randomized controlled trials for longer durations are necessary to determine whether cell-based tissue engineering offers long-term benefits to patients. It is also necessary to establish definitive protocols for stem/osteoprogenitor cell preparation and appropriate carrier

scaffolds for the cells that have optimal degradation and an osteoinductive surface.

4.2. Chair-side cellular grafting approach

The other approach to stem-cell-based bone regeneration relies on the direct use of a patient-derived fresh cellular graft prepared at the chair-side [101–106] or a commercially prepared allograft bone matrix that contains native MSCs (prepared from cadavers) [107,108] (Table 2). These procedures are relatively convenient for clinicians because they do not require laboratory support or extensive training.

In 2006, Smiler and Soltan [109] first reported a technique for chair-side cellular graft preparation using fresh aspirated bone marrow from the ilium that was mixed with a resorbable matrix, and they also showed bone marrow aspirate that was transplanted with biocompatible scaffolds [110] or allograft bone blocks [111] could successfully regenerate bone. Thereafter, cellular grafting approaches using the mononuclear fraction obtained from processed fresh marrow have been well documented. This method has been developed as a system called “Bone Marrow Aspirate Concentrate (BMACTM)”. The mononuclear fraction contains two principal lineages of stem cells: one responsible for hematopoiesis and another regarded as an MSC population [112].

The clinical effects observed in patients when using this approach have mainly been attributed to the presence of MSCs in the mononuclear cell fraction in the bone grafts

Table 1
Clinical studies of tissue engineering therapy for orofacial bone regeneration.

Authors (year) [Ref.]	Cell source	Cultivation period	Osteogenic induction	Constitution of grafts	Subject number	Surgical procedure
Schmelzeisen et al. (2003) [81]	Periosteum	4 passages	AS, Dex, β -gly, AsA (1 week)	PLGA fleece (Ethisorb [®]), fibrin glue (TissueColl [®])	2 patients	SL
Schimming and Schmelzeisen (2004) [82]	Periosteum	4 passages	AS, Dex, β -gly, AsA (1 week)	PLGA fleece (Ethisorb [®]), fibrin glue (TissueColl [®])	27 patients	SL
Yamada et al. (2004) [91]	Iliac BM	NA	FBS, Dex, β -gly, AsA	PRP, thrombin/calcium chloride	3 patients	ABA
Ueda et al. (2005) [214]	Iliac BM	NA	Serum, Dex, β -gly, AsA	PRP, β -TCP, thrombin/calcium chloride	6 patients	SL, ABA
Springer et al. (2006) [83]	Periosteum	3 weeks	AS, Dex, β -gly, AsA (1 week)	Collagen matrix	8 patients	SL
	Maxillary bone	2 weeks	AS (1.5 months)	BBM	2 patients	SL
Hibi et al. (2006) [92]	Iliac BM	4 weeks	AS, Dex, β -gly, AsA (1 week)	PRP, thrombin/calcium chloride	1 patient	ACO
Yamada et al. (2006) [36]	Iliac BM	1 month	Serum, Dex, β -gly, AsA	PRP, thrombin/calcium chloride	1 patient	Periodontal regeneration
Zizelmann et al. (2007) [84]	Periosteum	4 passages	AS, Dex, β -gly, AsA (1 week)	PLGA fleece (Ethisorb [®]), fibrin glue (TissueColl [®])	10 patients (14 sites)	SL
Ueda et al. (2008) [215]	Iliac BM	1 month	Serum, Dex, β -gly, AsA	PRP, thrombin/calcium chloride	14 patients	SL, ABA
Kulakov et al. (2008) [88]	Adipose tissue	3 passages	FBS, Dex, β -gly, AsA, 1,25-dihydroxy vitamin D ₃	Biodegradable matrix/PRP, thrombin/calcium chloride	8 patients	ABA
Meijer et al. (2008) [95]	Iliac BM	3 passages	FBS, Dex	HA (Pro Osteon [®] 500R)	6 patients	ABA, SL
Shayesteh et al. (2008) [96]	Iliac BM	NA	AS	HA/TCP	6 patients	SL
Beaumont et al. (2008) [85]	Periosteum	4 passages +1 week	AS, Dex, β -gly, AsA	PLGA fleece (Ethisorb [®]), fibrinogen (TissueColl [®]), thrombin, BBM	3 patients	SL
Yamada et al. (2008) [93]	Iliac BM	6 weeks	AS or FBS, Dex, β -gly, AsA	PRP, thrombin/calcium chloride	12 patients (16 sites)	SL
d'Aquino et al. (2009) [90]	Dental pulp	3 weeks	FBS	collagen sponge (Gingistat [®])	7 patients	SP
Mesimäki et al. (2009) [89]	Adipose tissue	2 weeks +2 days	AS	β -TCP/rhBMP-2	1 patients	ABA
Kaigler et al. (2010) [97]	Iliac BM	12 days	FBS, HS, hydrocortisone	Gelatin sponge (Gelfoam [®])	1 patient	SP
Voss et al. (2010) [86]	Periosteum	8 weeks	NA	PLGA fleece (Ethisorb [®])	35 patients	SL
	Iliac bone graft	–	–	Autologous iliac bone graft	41 patients	SL (control)
Lee et al. (2010) [99]	Iliac BM	4 weeks	FBS, Dex, β -gly, AsA	Freeze dried autologous bone, fibrin glue	1 patient	Reconstruction of large mandibular defect
Yamada et al. (2011) [94]	Iliac BM	6 weeks	AS or FBS, Dex, β -gly, AsA	PRP	23 patients	SL
Nagata et al. (2012) [87]	Periosteum	6 weeks	FBS, AsA	Particulate autologous bone/PRP	25 patients	ABA, SL
Kaigler et al. (2012) [100]	Iliac BM	12 days	FBS, HS, Hyd	Gelatin sponge (Gelfoam [®])	12 patients	SP
	–	–	–	Gelatin sponge (Gelfoam [®])	12 patients	SP
Behnia et al. (2012) [98]	Iliac BM	2 weeks	AS	HA/ β -TCP (ReproBone TM)/PDGF/plasma fibrin	3 patients (4 sites)	ACO

NA: not available; BM: bone marrow; AS: autologous serum; FBS: fetal bovine serum; HS: horse serum; Dex: dexamethasone; β -gly: β -glycerophosphate; AsA: L-ascorbic acid 2-phosphate; Hyd: hydrocortisone; PLGA: poly lactic-co-glycolic acid; PRP: platelet-rich plasma; TCP: tricalcium phosphate; HA: hydroxyapatite; BBM: bovine bone mineral (Bio-Oss[®]); SL: sinus lift; ABA: alveolar bone augmentation (onlay grafting); ACO: alveolar cleft osteoplasty; SP: socket preservation.

[102]. MSCs prepared by the chair-side method and combined with BBM particles have been shown to form lamellar bone and provide a reliable base for dental implants [103]. Rickert et al. [104] assessed in a prospective randomized clinical trial whether the bone formation was different after maxillary sinus floor elevation surgery using BBM (Bio-Oss[®]) mixed with autologous BMAC when compared with the use of autologous bone on the contralateral side. A histomorphometrical analysis revealed significantly more bone formation in the BMAC grafting group when compared with the autologous bone grafting group. Sauerbier et al. [105] demonstrated in a controlled, randomized, single-blinded clinical and histological trial that new bone formation after 3–4 months was equivalent in sinuses augmented with BMAC and BBM or a mixture of autologous bone and BBM. Thus,

this technique could be an alternative for the use of autografts to stimulate bone formation.

An interesting aspect of the chair-side method is that no clinical or histologic inflammatory response was observed after the operation [104,107]. The cells in freshly processed grafts are not completely homogeneous and may contain several cell types, such as MSCs, osteogenic cells, hematopoietic cells, angiogenic cells and stromal cells. Therefore, the freshly prepared cellular grafting material may behave somewhat similarly to a primitive bone niche to provide easier acceptance by the host environment without an unfavorable local inflammatory reaction. Furthermore, recent studies have demonstrated that BMSCs have a beneficial anti-inflammatory effect when administered directly to an injured tissue or intravenously [113–115] (see Section 7.1). Therefore, it is

Table 2

Clinical studies of stem-cell-based orofacial bone regenerative therapy using the chair-side cellular grafting approach.

Authors (year) [Ref.]	Cell source	Preparation method	Composition of grafts	Subject number	Surgical procedure
Smiler and Soltan (2006) [109]	Iliac BM	Fresh BMA	Resorbable matrix	3 patients	SL, SP
Smiler et al. (2007) [110]	Iliac BM	Fresh BMA	PepGen P-15 [®] , C-Graft [®] , β-TCP	5 patients	SL, ABA
Soltan et al. (2007) [111]	Iliac BM	Fresh BMA	Corticocancellous allograft bone block	5 patients	SL, ABA
Filho Cerruti et al. (2007) [101]	Iliac/sternum BM	MNC fraction	Autologous particulate bone, PRP	32 patients	ABA, SL
Wongchuensoontorn et al. (2009) [102]	Iliac BM	BMAC	Autogenous iliac bone graft, reconstruction bone plate	1 patient	Treatment of mandibular fracture
McAllister et al. (2009) [107]	Commercial product (from cadavers)	Ready to use	Stem cell-containing allograft (Osteocel [®])	5 patients	SL
Soltan et al. (2010) [216]	Iliac BM	Fresh BMA	HA (C-Graft [®]) or particulate bone	2 patients	ABA
Sauerbier et al. (2010) [103]	Iliac BM	FICOLL [®]	BBM, fibrin glue (TissueColl [®])	6 sites	SL
	Iliac BM	BMAC	BBM, autologous thrombin	12 sites	SL
Pelegine et al. (2010) [217]	Iliac BM	BMAC	None	13 patients (30 sites)	SP
Gonshor et al. (2011) [108]	Commercial product (from cadavers)	Ready to use	Stem cell-containing allograft (Osteocel [®])	18 patients (26 sites)	SL
Sauerbier et al. (2011) [105]	Iliac BM	BMAC	BBM, autologous thrombin	25 patients (34 sites)	SL
	Milled autogenous bone	–	BBM	11 patients (11 sites)	SL
Rickert et al. (2011) [104]	Iliac BM	BMAC	BBM	12 patients	SL (split-mouth design)
	Autogenous bone	–	BBM		
Schmelzeisen et al. (2011) [106]	Iliac BM	BMAC	BBM, autologous thrombin	1 patient	SL

BM: bone marrow; BMA: bone marrow aspirate; MNC: mononuclear cell; BMAC: bone marrow aspirate concentrate (BMAC[™] procedure pack; Harvest Technologies Corp., USA); FICOLL[®]: classic cell separation system using the synthetic polysaccharide method; PRP: platelet-rich plasma; TCP: tricalcium phosphate; HA: hydroxyapatite; BBM: bovine bone mineral (Bio-Oss[®]); SL: sinus lift; SP: socket preservation; ABA: alveolar bone augmentation (onlay grafting).

possible that the implanted naïve BMSC population plays an additional role in supplying specific growth/trophic factors to suppress over-inflammation in the local niche to further enhance bone regeneration. To improve this approach to stem cell therapy, it is necessary to elucidate the precise mechanisms underlying the enhancement of local bone regeneration by the implanted cells.

4.3. Cell-sheet-based tissue regeneration

Recently, a new tissue engineering technique, termed cell-sheet-based bioengineering, has been developed and utilized successfully for tissue regeneration [116–119]. In this technique, enzymatic cell digestion is not required and the cell-to-cell contact in the engineered construct thus remains intact, which should be beneficial for tissue regeneration. Additionally, ECM proteins that are secreted from the embedded cells can be used conveniently without requiring an additional scaffold. Several tissue engineering applications of cell sheets have been reported, *e.g.*, using the cell sheet to wrap a scaffold [120–122], using a multi-layered cell sheet [123,124], and using the cell sheet as a source of 3D pellet [125].

Cell sheet technology has now been utilized in the dental field to achieve efficient regeneration of periodontal and alveolar bone tissues. Ishikawa and his colleagues are the

pioneers in this field and first reported the fabrication of PDL cell sheets retrieved from culture on unique temperature-responsive culture dishes [126]. Thereafter, several pilot studies demonstrated that transplantation of PDL cell sheets regenerated periodontal tissue in experimental defect models in rats [127,128], dogs [124,126,129] and swine [130,131]. Tsumanuma et al. [132] reported using a canine severe defect model (one-wall intrabony defect) that transplantation of PDL cell sheets contributed greater periodontal regeneration with newly formed cementum and well-oriented PDL fibers than transplantation of iliac BMSC or alveolar periosteal cell sheets. Ishikawa's group also determined an optimal protocol for the extraction, expansion and characterization of human PDL cells [133] and validated the safety and efficacy of the PDL sheet for clinical trials [134]. A clinical trial study using the PDL sheet technology is currently under way for regenerative periodontal therapy [135].

Periosteum-derived cells have also been used as a cell sheet source for bone regenerative therapy. The human cultured periosteal sheet is an osteoinductive biomaterial, even without the inclusion of conventional scaffold materials [136]. Nagata et al. [87] demonstrated that the use of a cultured autologous periosteal cell sheet for sinus lifting induced bone remodeling that may enable the regeneration of bone tissues with complex morphology in a wide area and thus expand indications for dental implants. Dental follicle cells (DFCs) have also been

suggested as a good candidate for cell-sheet-based periodontal [137] and root [121] regeneration.

5. Factors that influence stem cell-based regenerative therapy

Stem-cell-based therapy has shown promise for orofacial bone regeneration but is a relatively new technology, and the events following transplantation are poorly understood. In clinical assessments of stem-cell-mediated ridge augmentation, it is not clear whether the new bone formation was provided by the surviving implanted cells (osteoinduction) or host osteogenic cells (osteoconduction) [138]. Although BMSCs have been extensively characterized during culture expansion, relatively little is known of their biological properties in the naïve state. In addition, culture expansion of BMSCs may alter their fundamental biological properties, which may affect the immune responses by the recipient immune cells. In this section, factors that affect stem cell-based bone regeneration related to the survival of the transplanted cells, pre-culture conditions and possible local immune response are discussed.

5.1. Survival of transplanted cells

Successful bone tissue engineering by cell transplantation requires sufficient numbers of cells with osteogenic capacity and retention of cellular viability to permit the transplanted cells to produce an ECM for the tissue regeneration. Indeed, the implantation of a high dose of cells resulted in a significantly higher amount of newly formed bone when compared with low and medium doses in a rabbit calvarial vertical bone regeneration model [139]. However, the fate of the transplanted cells and its effect on clinical outcome is uncertain. Meijer et al. [95] histologically examined biopsies taken 4 months after reconstruction of an intra-oral bone defect by transplantation of autologous BMSCs and HA particles, and observed *de novo* bone formation by the transplanted cells in only 1 of 3 patients.

Several animal studies suggest that transplanted cells die quickly or migrate out of the transplanted site. When fluorescently labeled MSCs that were seeded on a HA/TCP scaffold were subcutaneously implanted in isogenic rats, the number of transplanted cells gradually decreased, and the donor cells could no longer be identified fourteen days after implantation [140]. In a goat model, viable MSCs, which were transplanted using a gelatin carrier into osteochondral defects, were identified until 2 days after implantation, whereas an extensive loss of the implanted MSCs occurred by days 7 and 14, possibly because of the fragmentation, dislodgement, death and passive migration of the cells [141]. Boukhechba et al. [142] tracked donor and recipient cells after implantation of BMSCs in an isogenic model of ectopic bone formation using Y-chromosome *in situ* hybridization and showed that the grafted cells did not survive more than three weeks after implantation. Although some of the cells migrated to peripheral lymphoid organs, the grafted BMSCs triggered new bone

matrix formation through the attraction of recipient cells into the implants.

Tasso et al. [143] investigated the host response to the implantation of BMSCs in a porous ceramic scaffold in a mouse subcutaneous model and demonstrated that two different waves of cells (CD31⁺ endothelial progenitors and CD146⁺ pericyte-like cells) migrated from the host to the BMSC-seeded ceramic to participate in the development of the newly formed tissue. Survival of the transplanted cells can be supported by sufficient vascular supply; therefore, the cross-talk between implanted exogenous BMSCs and resident stem/progenitor cells may play a pivotal role in increasing vascularization in BMSC implants to support cell survival and subsequent bone regeneration. This cross-talk may involve the cell-mediated immune response (see Section 5.3). Further studies on host-donor cross-talk help to elucidate the cell interactions that occur during bone regeneration and provide innovative approaches for advanced cell-based bone regeneration therapy.

5.2. Pre-culture condition of the donor cells

Effects of the pre-culture condition of transplanted cells on *in vivo* bone formation have been extensively studied. It appears that human BMSCs lose their *in vivo* osteogenic ability during *in vitro* expansion using classic culture methods regardless of the length of osteogenic induction [144]. Serum-free culture using a serum substitute (Ultrosor[®] G [145] or StemPro[®] [146]) allows better expansion of human BMSCs, and BMSCs expanded in serum-free medium showed ectopic bone formation as efficient as that obtained with BMSCs expanded in conventional serum-containing medium [146]. Although CaP-based biomaterials have significant potential for bone regeneration, pre-culture of human periosteum-derived cells with biomimetic calcium and phosphate supplementation resulted in partial or complete abrogation of *in vivo* ectopic bone formation [147].

The duration of *in vitro* pre-culture is a critical factor in the ability of BMSCs to regenerate bone. Agata et al. [144] reported that 2-week osteogenic induction of human BMSCs increased the probability of success in ectopic bone formation when compared with 1-week induction. In contrast, Castano-Izquierdo et al. [148] reported that osteogenic induction of rat BMSCs for only 4 days resulted superior *in vivo* bone formation than induction for 16 days. Niemeyer et al. [149] evaluated the survival of undifferentiated and osteogenically induced human bone marrow- or adipose tissue-derived MSCs after transplantation in immunocompetent mice. Undifferentiated MSCs were detected in the majority of cases; however, osteogenically induced MSCs were only detected in a few cases, which suggests that osteogenically induced MSCs were eliminated by the host immune system. These reports suggest that the optimum pre-culture conditions for human BMSCs to maintain their survival at the transplantation site for stable bone regeneration remain unclear and controversial. Thus, optimal pre-culture conditions should be established when designing protocols for stem-cell-based bone regeneration.

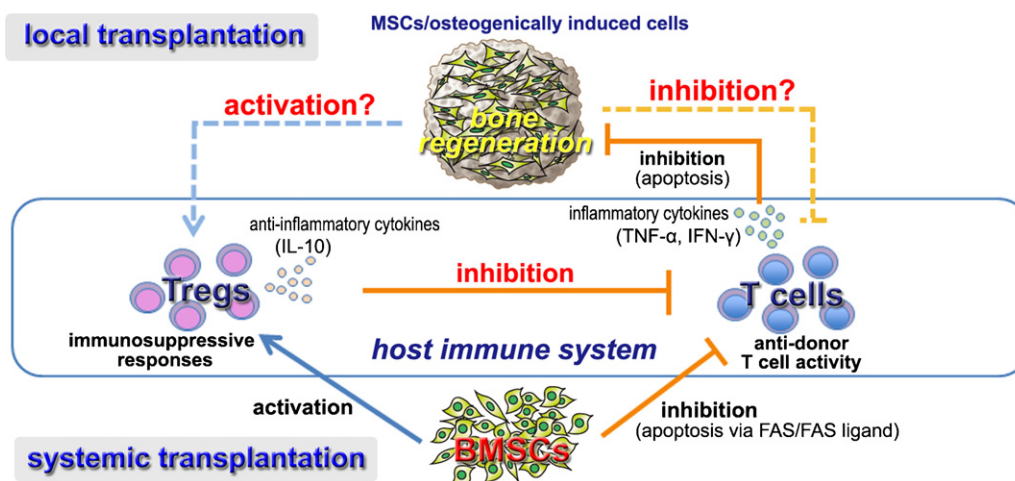


Fig. 4. Schematic diagram illustrating the cross-talk between grafted cells and microenvironment in bone regeneration. The clinical success of bone regeneration may be significantly affected by the host immune system. Inflammatory cytokines, such as TNF- α and IFN- γ , secreted from T cells strongly inhibit bone regeneration by inducing apoptosis of the grafted cells [150]. In contrast, regulatory T cells (Tregs) enhance bone regeneration by inhibiting the activation of T cells using anti-inflammatory cytokines such as IL-10. Although systemically transplanted bone marrow-derived mesenchymal stem/stromal cells (BMSCs) also inhibit the recipient immune system by activating recipient Tregs or inhibiting the activation of T cells [155], it is still unclear whether locally grafted MSCs/osteogenically induced cells have the same effect on the local immune system.

5.3. Local immune responses to cellular grafting

It is known that ectopic bone formation by stem cells transplanted in animal models is not always predictive of the clinical outcomes for orthotopic bone formation in humans [95]. One possible reason for this discrepancy is that most animal studies use immune-compromised mice, which lack some components of the immune system (*e.g.*, T cells and B cells) present in typical patients. Recently, involvement of the recipient immune system in BMSC-mediated bone regeneration has attracted considerable attention.

Liu et al. [150] reported that BMSC-mediated bone regeneration is partly controlled by the host local microenvironment in which immune cells and inflammatory cytokines affect the BMSCs. Autologous BMSCs did not generate bone when the cells were subcutaneously transplanted into wild-type mice using an HA/TCP carrier, whereas abundant bone formation was observed when BMSCs were transplanted into immune-compromised (T cell-deficient) mice. In addition, when T cells derived from wild-type mice were systemically injected into the immune-compromised mice prior to BMSCs transplantation, bone formation was significantly inhibited, with increased production of IFN- γ and TNF- α by host CD4⁺ T cells that may induce BMSC apoptosis. Furthermore, Liu et al. demonstrated that calvarial bone regeneration in wild-type mice could be enhanced through suppression of IFN- γ and TNF- α (*i.e.*, inhibition of host T cell activity) using systemic infusion of regulatory T cells (Tregs) or site-specific aspirin treatment. However, Ren et al. [151] reported that the inflammatory niche of IFN- γ together with TNF- α , IL-1 α or IL-1 β activates the immunosuppressive ability of BMSCs to provoke the expression of high levels of several chemokines and inducible nitric oxide synthase. The chemokines drive T cell migration towards the BMSCs, where T cell responsiveness is suppressed by nitric oxide (NO). These

reports indicate that the environmental immune status at the grafting site substantially affects BMSC-mediated bone regeneration.

Conversely, donor BMSCs produce various anti-inflammatory factors to inhibit the proliferation and function of several types of immune cells [152]. Although the overall outcome of MSC-mediated immunosuppression is inhibition of T cell activation and proliferation, MSCs have also been shown to induce T cell differentiation into immunosuppressive Tregs [153,154]. In addition, systemically transplanted MSCs induce recipient T cell apoptosis and resulting increase in the number of Tregs [155]. MSCs can also stimulate macrophages and dendritic cells to secrete IL-10 [153,156], which in turn has a profound immunosuppressive effect on T cells.

Animal studies have demonstrated that allogenic β -islet [157] or heart [158] transplantation coupled with MSC infusion results in successful engraftment of the MSCs and reduced rejection of the transplanted organs. Both studies indicate that MSC co-transplantation prolongs graft survival, possibly through impaired anti-donor T cell activity and expansion of IL-10-secreting Tregs [157,158]. It has also been demonstrated that xenogenic transplantation of human BMSCs results in poorer bone regeneration than autologous transplantation of ovine BMSCs in a critical-size tibia defect [159].

These findings strongly suggest that the cross-talk between implanted donor BMSCs and recipient immune cells plays a key role in determining the success of BMSC-mediated tissue regeneration (Fig. 4); therefore, the local immune response to BMSC transplantation should be evaluated in future studies. The knowledge of native BMSC biology and interactions of BMSCs with their microenvironment, *i.e.*, the stem cell niche, in healthy or regenerating bone tissues will provide guidance for future clinical applications.

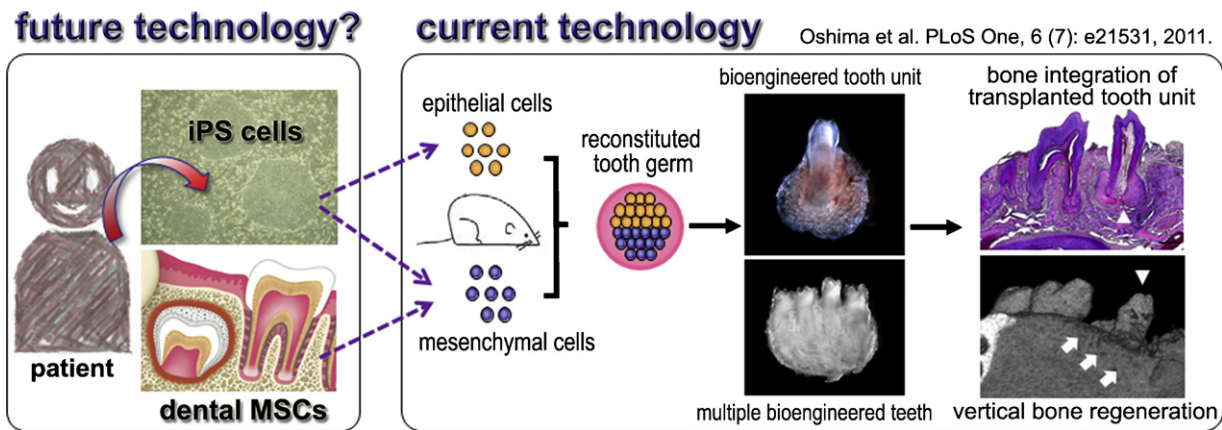


Fig. 5. Schematic representation of the current regenerative strategy for mature tooth/organ replacement. Recent advances in biotechnology have enabled the fabrication of a bioengineered tooth unit (whole tooth and periodontal tissues surrounded by alveolar bone) and multiple arranged tooth units from mouse tooth-germ-derived single epithelial cells and mesenchymal cells. Upon transplantation, the bioengineered tooth unit (arrowheads) was engrafted in the alveolar bone defect of the recipient mouse *via* bone integration, which resulted in vertical bone formation (arrows). Future stem-cell technology may permit the development of bioengineered tooth units using patient-derived iPS cells or dental mesenchymal stem/stromal cells (MSCs). The right panel was reproduced from Oshima et al. [166] under the open-access license policy of PLoS One.

6. Pre-clinical studies on complex oral tissue/organ regeneration

Regeneration technologies for complex oral tissues/organs, such as the teeth, salivary glands, mandible condyle and tongue, have not yet reached the clinical trial stage because of their developmental and structural complexity. However, recent advances based on animal research have identified feasible strategies to regenerate these tissues/organs.

6.1. Tooth/root regeneration

The ultimate goal of tooth regeneration is to develop fully functioning bioengineered teeth that can replace lost teeth [160]. In contrast, the regeneration of the tooth root is a conceivably more realistic and clinical applicable approach, especially for prosthodontists, because the regenerated tooth root can be used as an abutment tooth to permit fixed-prosthetic approaches, such as crown and bridge treatments. Sonoyama et al. [161] demonstrated that a root/periodontal complex constructed using PDL stem cells (PDLSCs), stem cells from the apical papilla (SCAP) and a HA/TCP scaffold, was capable of supporting an artificial crown to provide normal tooth function in a swine model. In addition, cell sheet technology using DFCs in combination with a dentin matrix-based scaffold has been applied successfully to tooth root reconstruction [121]. New stem-cell-based technology for the regeneration of the tooth root and its associated periodontal tissue may offer clinical opportunities for the treatment of damaged or lost teeth.

Regeneration of the entire tooth is expected to be one of the highest achievements in the field of dentistry. Tooth engineering to form dental structures *in vivo* has been established using many different types of stem cells from mice [162], rats [163] and pigs [164]. Ikeda et al. [165] demonstrated a fully functioning tooth replacement in a mouse through the transplantation into the alveolar bone of

bioengineered tooth germ reconstituted from epithelial and mesenchymal progenitor/stem cells in a collagen gel. The bioengineered tooth, which was erupted and occluded, had the correct tooth structure, hardness of mineralized tissues for mastication, and response to noxious stimulation such as mechanical stress and pain in cooperation with other oral and maxillofacial tissues. Using the same cell source used for the bioengineered tooth, the *in vivo* reconstruction of a murine “bioengineered tooth unit” was recently demonstrated [166] (Fig. 5). Surprisingly, the unit comprised not only a mature tooth and periodontal ligament but also alveolar bone. The unit provided a fully functional tooth with vertical bone regeneration when the unit was transplanted into a vertical alveolar bone defect in a mouse model. These findings resulted in a new concept in tooth regeneration therapy: the transplantation of a bioengineered tooth has great potential for not only whole-tooth regenerative therapy but also as a treatment in clinical cases where tooth loss is accompanied by a serious alveolar bone defect [166]. One of the major hurdles in the clinical application of tooth regeneration technology is the identification of an appropriate autologous stem cell source in humans. In this regard, iPS cells may be an appropriate cell source because they can be differentiated to dental epithelial and mesenchymal cells [167,168] and can be prepared from the patients’ own somatic cells.

6.2. Salivary gland regeneration

Regeneration of salivary glands by stem cell transplantation is an important study topic for head and neck oncology and surgery because radiotherapy unavoidably impairs salivary gland function and results in xerostomia (dry mouth syndrome) as a side effect. Two main regenerative approaches have been applied to functionally restore damaged salivary glands. One approach is to develop an artificial salivary gland using tissue engineering technologies [169–171]. Another approach is to apply stem cells to the damaged salivary gland tissue. In a

mouse model, adipose-derived MSCs transplanted in irradiated submandibular glands restored salivary gland function [172]. Transplantation of BMSCs into the mouse tail vein also repaired the function of irradiated salivary glands [173]. Recently, primitive salivary gland stem cells were isolated from mice, and intra-glandular transplantation of these cells successfully repaired the function of irradiated salivary glands [174,175]. These reports suggest that stem cell transplantation may be used to functionally repair damaged salivary glands. The detailed regeneration mechanism should be clarified, *i.e.*, whether the donor stem/progenitor cells repair damaged host cells through replacement or by activating turnover of the host cells.

6.3. Mandible condyle regeneration

Damage to the temporomandibular joint disc or condyle (condylar osteochondral defect) arising from trauma or arthritis can result in lifelong pain and disturbed masticatory function for patients. Tissue regeneration strategy on these defects can hold promise to affect the quality of life (QOL) of these patients. In a goat model, the combination of cartilage tissue engineering using cartilage-derived progenitor cells carried in a hydrogel and distraction osteogenesis was successfully used to reconstruct condylar osteochondral defects [176]. Additionally, a human-shaped mandibular condyle was successfully engineered from chondrogenically and osteogenically induced rat BMSCs encapsulated in a biocompatible polymer [177,178]. BMSCs that were induced to differentiate into chondrogenic and osteogenic cells produced regeneration of rabbit mandibular condyle that was enhanced by low-intensity pulsed ultrasound [179]. These findings may provide an initial proof of concept for the ultimate stem-cell-based tissue engineering of degenerated articular condyles in the context of diseases such as rheumatic arthritis.

6.4. Tongue regeneration

Loss of tongue tissue from surgical resection can profoundly affect the QOL because the tongue plays a critical role in speech, swallowing and airway protection. Therefore, reconstruction of tongue defects has been a continuing challenge in dentistry. Cell-based reconstruction of the tongue was reported in a rat model where myoblast/progenitor cells carried in a collagen gel were implanted into the hemiglossectomized tongue [180,181] to provide successful muscle regeneration in the tongue with reduced scar contracture [180]. The tongue is a complex structure that includes skeletal muscle fibers, mucosa with taste buds, and nervous tissue; therefore, functional regeneration is difficult. Egusa et al. [182] demonstrated that applying of cyclic strain to BMSCs greatly accelerated *in vitro* skeletal myogenesis to achieve aligned myotube structures, suggesting the importance of cellular alignment for creating physiologically relevant environments to engineer skeletal muscle. Advances in stem cell biology and tissue engineering may enable the reconstruction of the damaged or resected tongue with normal physiological function.

7. Cell-based immunotherapy using MSCs

Conventionally, MSCs have been recognized as a type of grafting material that can cooperatively fill tissue defects with scaffolds at a local site. However, a new role of MSCs as immune modulators was recently revealed, and the potential usage of MSCs has been expanded to the treatment of immune-mediated diseases.

7.1. Systemic delivery of BMSCs for immune-mediated diseases

Originally, BMSCs were used as feeder cells to expand hematopoietic stem cells (HSCs) *in vitro*, and recent studies revealed that BMSCs constitute an essential HSC niche component in bone marrow [183]. However, MSCs isolated from adult tissue mediate tissue and organ repair, and they also home to the site of injury, where they secrete cytokines and growth factors that participate in repair processes including the proliferation and differentiation of endogenous progenitor cells [184]. It is therefore likely that inflammatory cytokines, such as TNF- α , at the inflammatory site stimulate the homing of endogenous or transplanted MSCs [185]. Furthermore, MSCs express matrix metalloproteinase to invade through ECM barriers [186]. Although the mechanism underlying MSC homing is still not clear, it appears to be very similar to that of leukocytes, as it involves steps such as tethering, rolling, and transmigration at the wound site [187,188].

Notably, recent studies demonstrated that systemically transplanted BMSCs exhibit a profound immunomodulatory effect on immune cells and may thus be used as a therapy for immune-mediated diseases [189,190]. In this scenario, systemic administration of BMSCs induces peripheral tolerance, and the BMSCs then migrate to injured tissues, where they inhibit the release of pro-inflammatory cytokines and promote the survival of damaged cells [190]. The immunomodulatory effects of BMSCs have been examined in a various animal models of immune-mediated inflammatory diseases, including rheumatoid arthritis [191], osteoporosis [192], diabetes [193], acute renal failure [194], acute lung injury [195] and systemic lupus erythematosus (SLE) [196]. In addition, an immunosuppressive effect of infused MSCs in patients has been successfully shown in graft-versus-host disease (GVHD) [197] and refractory inflammatory bowel disease [198]. However, the specific mechanism underlying the immunomodulatory effect of MSCs is still unclear; therefore, many questions need to be addressed before the therapeutic promise of these cells can be realized.

7.2. Possible applications of the MSC-based immunotherapy in dentistry

Several reports have revealed that stem cells derived from oral tissues possess unique immunomodulatory properties. Ding et al. [130] transplanted allogeneic PDLSC sheets into a pig periodontitis model and demonstrated low immunogenicity and marked immunosuppressive function exerted

viaprostaglandin E2 (PGE2)-induced T-cell anergy. PDLSCs, SCAP and dental pulp stem cells have also been reported to possess *in vitro* immunosuppressive properties [199–201]. The immunoregulatory characteristics of these dental stem cells may provide new therapeutic strategies, such as allogeneic stem-cell-based therapies and the treatment or prevention of T cell alloreactivity in allogeneic transplantation.

Other MSC-based immunotherapeutic strategies in dentistry involve the systemic delivery of MSCs including dental MSCs. The predominance of tissue-destructive IL-17-producing Th17 cells and decreased number and function of tissue-protective Tregs has been confirmed in various inflammatory states, including autoimmune disease [202]. Yamaza et al. [203] demonstrated that stem cells from human exfoliated deciduous teeth (SHED) inhibit Th17 cell differentiation, whereas they increase the number of Tregs *in vitro*. Yamaza et al. also demonstrated that systemic SHED transplantation improved SLE phenotypes in the SLE mouse model, which showed an increase in the ratio between Tregs and Th17 cells. Kikuri et al. [204] demonstrated that systemic infusion with allogeneic BMSCs prevented and cured bisphosphonate-related osteonecrosis of the jaw (BRONJ)-like disease in mice, possibly *via* the induction of peripheral tolerance, which was shown as an inhibition of Th17 and increase in the number of Treg cells. Zhang et al. [205] demonstrated that cell-based therapy using a systemic infusion of gingiva-derived MSCs ameliorated experimental colitis in mice by suppressing inflammatory cell infiltration and proinflammatory cytokine secretion as well as by increasing Treg accumulation and IL-10 expression at local intestinal sites. El-Menoufy et al. [206] demonstrated that oral ulcer healing was accelerated by the injection of autologous BMSCs around chemically induced oral ulcers in the oral cavity of dogs. It was concluded that the beneficial effects of BMSCs may be mediated through the induction of angiogenesis together with increased ECM formation; however, immunomodulatory effects of BMSCs may also be involved. The therapeutic effects of systemic BMSC transplantation on impaired salivary gland function [173] may also be mediated by the immunomodulatory effects of MSCs.

These new immunomodulatory properties of MSCs will attract the attention of dental scientists not only to the differentiation of MSCs for regenerative therapy but also to the possible application of MSCs to immunotherapy. In addition, the concept of the MSC-based immunomodulation may be applicable to suppression of the local immune response during transplantation to achieve optimal tissue regeneration.

8. Dental stem cell banking

Growing evidence has demonstrated that dental tissues are a rich source of MSCs [1]. Dental stem cells may be useful for regenerative and immune therapies in medical fields [203,205,207]. A recent animal study demonstrated that human dental-pulp-derived stem cells may provide greater therapeutic benefit for treating spinal cord injury than human BMSCs [207]. However, the use of a patient's own dental-tissue-derived stem cells at the time of therapeutic necessity has serious

limitations because it would require the extraction of a remaining tooth. Dental stem cell banking, *i.e.*, the process of storing stem cells obtained from patients' deciduous teeth and wisdom teeth, may be one strategy to realize the potential of dental-stem-cell-based regenerative therapy [208–210].

Recently, cell/tissue banks in the dental field have been planned and placed into practice in several countries, *e.g.*, Advanced Center for Tissue Engineering Ltd., Tokyo, Japan (<http://www.acte-group.com/>); Teeth Bank Co., Ltd., Hiroshima, Japan (<http://www.teethbank.jp/>); Store-A-Tooth™, Lexington, USA (<http://www.store-a-tooth.com/>); BioEDEN, Austin, USA (<http://www.bioeden.com/>) and Stemade Biotech Pvt. Ltd., Mumbai, India (<http://www.stemade.com/>). Once stem-cell-containing tissues, such as PDL, pulp tissues, apical papilla, or the tooth itself, are obtained from the patient, they can be cryopreserved for many years to retain their regenerative potential [201,211,212]. Dental stem cells can be isolated from the cryopreserved tissue/tooth whenever required for future regenerative therapies [208,210,213]. These autologous stem cells given to a patient would be recognized as host cells and should therefore be tolerated by the immune system

Although successful autologous transplantation of banked teeth has been achieved in the clinic (<http://www.teethbank.jp/>), stem-cell-based tissue engineering therapies using stem cell banking have not yet been reported. Therefore, the utility of stem cell banking in dentistry should be carefully evaluated. In addition, legislation for the banking system is necessary because it provides bio-insurance for a future use that is highly unlikely. Checks and audits must be conducted to determine whether the banking company can operate well into the future, and whether the cryopreserved cells and tissues are maintained in good quality for future use in transplantation.

9. Conclusion

We have entered a new era in the regeneration of orofacial bone, where molecular enhancement by osteoinductive materials and stem-cell-based therapies can be used to improve and expedite clinical outcomes. Current active research areas of stem-cell-based therapy in dentistry are focused on tissue engineering and chair-side cellular grafting approaches that may result in more predictable regenerative outcomes in the future. More intensive basic and translational research is necessary, and clinical randomized controlled trials with long durations should be performed to advance the field using scientific evidence that can ultimately offer long-term benefits to patients.

Local immune responses by the host cells against the grafting materials are highly relevant in tissue engineering and regenerative medicine. We believe that a complete understanding of biological processes on both donor and recipient sides during bone regeneration is crucial to design new and more effective clinical strategies for stem-cell-based bone regeneration. In addition, the recently observed immunomodulatory function of MSCs may be applicable to strategies of how to suppress the local immune response during transplantation to achieve optimal tissue regeneration.

Conventionally, individuals working in the prosthodontic field rarely perform basic biological studies. However, the increased requirement for new technologies for implant dentistry is encouraging prosthodontists to be involved in or at least understand regenerative medicine, including stem cell biology. Based on the accumulated laboratory and clinical evidence, a road map to establish “stem-cell-based dentistry” should thus be presented by authorized organizations, including those related to the field of prosthodontics, as a solid consensus toward the future of dentistry.

Conflict of interest statement

All authors state that they have no conflict of interest.

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