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Review

The Regenerative Medicine in Oral and Maxillofacial Surgery: The Most Important Innovations in the Clinical Application of Mesenchymal Stem Cells

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Abstract

Regenerative medicine is an emerging field of biotechnology that combines various aspects of medicine, cell and molecular biology, materials science and bioengineering in order to regenerate, repair or replace tissues.

The oral surgery and maxillofacial surgery have a role in the treatment of traumatic or degenerative diseases that lead to a tissue loss: frequently, to rehabilitate these minuses, you should use techniques that have been improved over time. Since 1990, we started with the use of growth factors and platelet concentrates in oral and maxillofacial surgery; in the following period we start to use biomaterials, as well as several type of scaffolds and autologous tissues. The frontier of regenerative medicine nowadays is represented by the mesenchymal stem cells (MSCs): overcoming the ethical problems thanks to the use of mesenchymal stem cells from adult patient, and with the increasingly sophisticated technology to support their manipulation, MSCs are undoubtedly the future of medicine regenerative and they are showing perspectives unimaginable just a few years ago. Most recent studies are aimed to tissues regeneration using MSCs taken from sites that are even more accessible and rich in stem cells: the oral cavity turned out to be an important source of MSCs with the advantage to be easily accessible to the surgeon, thus avoiding to increase the morbidity of the patient.

The future is the regeneration of whole organs or biological systems consisting of many different tissues, starting from an initial stem cell line, perhaps using innovative scaffolds together with the nano-engineering of biological tissues.

Key words: Regenerative medicine; Mesenchymal Stem Cells; Bone regeneration; Dental Pulp Stem Cells; human Periapical Cysts Mesenchymal Stem Cells; hPCy-MSCs.

Introduction

Regenerative medicine is an emerging field of biotechnology that combines various aspects of medicine, cell and molecular biology, materials science and bioengineering in order to regenerate, repair or replace tissues.

The oral surgery and maxillofacial surgery have a role in the treatment of traumatic or degenerative diseases that lead to a tissue loss: frequently, to rehabilitate these minuses, you should use techniques that have been improved over time. Since 1990, tissue engineering has developed protocols in which it has been proposed the use of platelet concentrates, which showed enormous benefits for the patient: they favored and accelerated the post-surgical and provided

a support for tissue regeneration due to growth factors contained in them. Several authors [1-4] have described the importance of growth factors in tissue repair processes, in fact, they are important elements for new tissue production, moreover, they perform feedback controls on inflammatory processes within the tissue graft, in cases of regenerative surgery.

Whitman[5] and Marx[6] published the first studies on the use of growth factors contained in platelet gel, called Platelet-Rich Plasma (PRP).

Thanks to Marx's studies, it was possible to verify that the platelet concentrate is a very effective tool for the modulation of wound healing and tissue regeneration. However, the PRP showed a number of disadvantages, such as the need of having to run a complex and expensive protocol for its production. To overcome some of these problems, the PRGF (Plasma Rich in Growth Factors) was introduced in the list of platelet concentrates. The PRGF is considered an evolution of the PRP [7,8] and it allows a higher concentration of growth factors in platelet preparation. Among the advantages of the PRGF, we can cite the lesser amount of blood taken for the preparation and a procedure relatively faster, while, among the disadvantages we can mention the rapid clot formation, which require speed in its surgical use.

In 2001, Choukroun *et coll.* have instead proposed an alternative technique: the PRF (Platelet Rich Fibrin). PRF is derived from a simple preparation protocol that does not require alteration of the blood; it is a platelet concentrate rich in GFs that contains a three-dimensional matrix of autologous, elastic and flexible fibrin.

Dohan *et al*. have shown that platelet cytokines (PDGF, TGFbeta1 and IGF-1) are present in three-dimensional fibrin matrix derived from these platelet concentrates; moreover, PRF matrix traps glycosaminoglycans such as heparin and hyaluronic acid, which have considerable affinity with some peptides present in the bloodstream and therefore show strong ability of chemotaxis and diapedesis, useful for the healing of tissue damaged, for example, by trauma [9]. Moreover, it was shown that this matrix can be a valuable support for the transplantation of bone morphogenetic proteins (BMP) issued in a progressive manner to induce osteogenic differentiation, as demonstrated by recent studies on muscle preparations[10,11]; about this, the results of Wiltfang *et al*. are encouraging, in fact, they show an improvement of osteoblast proliferation in cases in which it was used the PRF compared to PRP [12].

Marrelli et al. described a case in which is documented the filling with PRF of a large osteolytic cavity and complete bone reformation [13]. Tatullo *et al*. have suggested that the osteoinductive potential of PRF is related to its neoangiogenic ability and concentration of GFs, in relation to the fibrin content and platelet cytokines present, all suitable for the totipotent cell migration and activation of pre-osteoblastic cells present in the surgical site, fundamental aspects for bone regeneration [14].

Platelets concentrates are, thus, versatile products in surgery, with regard to their biological properties and their easy manipulation in the form of gel or membranes; these features allow the use of PRF as well as other platelet concentrates in cases, for example, of maxillary surgical sites or in the surgery of maxillary sinus [15].

The frontier of regenerative medicine nowadays is represented by the mesenchymal stem cells (MSCs): overcoming the ethical problems thanks to the use of mesenchymal stem cells from adult patient, and with the increasingly sophisticated technology to support their manipulation, MSCs are undoubtedly the future of medicine regenerative and they are showing perspectives unimaginable just a few years ago. Most recent studies are aimed to tissues regeneration using MSCs taken from sites that are even more accessible and rich in stem cells: the oral cavity turned out to be an important source of MSCs with the advantage to be easily accessible to the surgeon, thus avoiding to increase the morbidity of the patient.

Mesenchymal stem cells of oral origin

The aim of the regenerative medicine and tissue engineering is to regenerate and repair the damaged cells and tissues in order to establish the normal functions [16]. The regenerative medicine involves the use of biomaterials, growth factors and stem cells [17]. Regeneration of the tissues exists naturally due to the presence of stem cells with the potential to self-regenerate and differentiate into one of more specialized cell types. However, this regenerative potential decreases with age and regeneration is not sufficient to repair the damages produced by degenerative, inflammatory or tumor based diseases[18]. Stem cells are immature and unspecialized cells with the ability to renew and divide themselves indefinitely through "self-renewal" and able to differentiate into multiple cell lineages [19]. The stem cells use for regenerative medicine should fit the following criteria: they can be: *a)* found in abundant numbers and can be differentiated in multiple cell lineages in a reproducible and controllable manner; *b)* isolated by minimally invasive procedure with minimal morbidity for patients, *c)* produced in accordance with GMP (Good manufacture Practice) and *d)* transplanted safely [20,21]. In the last decade, several improvements have been produced in the comprehension of stem cells properties in view of the fact that these cells have an important role in the repair of

every organ and tissue.

In general, the stem cells are divided into three main types that can be utilized for tissue repair and regeneration: *i)* the embryonic stem cells derived from embryos (ES) [22,23]; *ii)* the adult stem cells that are derived from adult tissue [24]; and *iii)* the induced pluripotent stem (iPS) cells that have been produced artificially via genetic manipulation of the somatic cells [25]. ES and iPS cells are considered pluripotent stem cells because they can develop into all types of cells from all three germinal layers. Both stem cells have technical and moral obstacles, in addition these cells are not easy to control and they can form tumors after injection[22]. On the contrary, adult stem cells are multipotent because they can only differentiate into a restricted number of cell types. Adult stem cells, also termed postnatal stem cells or somatic stem cells, are discovered in a particular area of each tissue named "stem cell niche."

Different type of postnatal stem cells resides in numerous mesenchymal tissues and these cells are at the same time referred to as mesenchymal stem cells (MSCs) [24,26]. MSCs were first isolated and characterized from bone marrow (BMSCs) by Friedenstein *et al.* in 1974 [27]. Subsequently, different studies have showed that MSCs can be isolated from other tissues, such as peripheral blood, umbilical cord blood, amniotic membrane, adult connective, adipose and dental tissues[28-32].

Recently, orofacial and dental tissues have acquired interest as a further accessible source of mesenchymal stem cells [33] due to the fact that the oral area is rich in MSCs (**Table 1**). Today, every cell population which has the following characteristics independently of its tissue source, is usually referred as MSCs: *i)* they adhere to plastic and have a fibroblast-like morphology; *ii)* they have the capacity of self-renewal and could differentiate into cells of the mesenchymal lineage such as osteocytes, chondrocytes and adipocytes. In addition, MSCs also can also differentiate, under appropriate conditions, into cells of the endoderm and ectoderm lineages such as hepatocytes and neurons, respectively [34,35]. Phenotypically, MSCs express the CD13, CD29, CD44, CD59, CD73, CD90, CD105, CD146 and STRO-1 surface antigens, and they do not express CD45 (leukocyte marker), CD34 (the primitive hematopoietic progenitor and endothelial cell marker), CD14 and CD11 (the monocyte and macrophage markers), CD79 and CD19 (the B cell markers), or HLA class II [36]. Research related to MSC from oral origin began in 2000 [37] and every year numerous investigations have demonstrated that oral tissues, which are simply available for dentists, are a rich source for mesenchymal stem cells [33,38].

Today numerous types of MSCs have been isolated from teeth: in 2000 MSCs were first isolated by Gronthos *et al.* from dental pulp (DPSCs) [37,38]. These cells possess phenotypic characteristics similar to those of BMSCs [39], and they have definitive stem cell properties such as self-renewal and multi- differentiation capacity, and can form the dentin-pulp structure when transplanted into immunocompromised mice [40]. Moreover, DPSCs participate in the regeneration of non-orofacial tissues, in fact, these cells have been differentiated into hair follicle-, hepatocyte-, neuron-, islet-, myocyte- and cardiomyocyte-like cells [41-46]. Subsequently, MSCs have been also isolated from dental pulp of human exfoliated deciduous teeth (SHEDs). These cells, like DPSCs, have the ability to differentiate *in vitro* in odontoblasts, osteoblasts, adipocytes and neuron-like cells. Also SHEDs were able to form dentin and bone when transplanted with HA/TCP *in vivo*[47].

Table 1: Mesenchymal Stem Cells from dental tissues

Name	Site	Date of discover	Authors	Country	Institution
DPSCs	Dental Pulp	2000	S. Gronthos, M. Mankani, J. Brahim, P.G. Robey, S. Shi	USA. Bethesda, Maryland	National Institute on Dental Research, National Institutes of Health
SHED	human Exfoliated Deciduous Teeth	2003	M. Miura, S. Gronthos, M. Zhao, B. Lu, L.W. Fisher, P. G. Robey, S. Shi	USA. Bethesda, Maryland	National Institute on Dental Research, National Institutes of Health
PDLSCs	Periodontal Ligament	2004	B. M. Seo, M. Miura, S. Gronthos, P.M. Bartold, S. Batouli, J. Brahim, M. Young, P.G. Robey, C.Y. Wang, S. Shi	USA. Bethesda, Maryland	National Institute on Dental Research, National Institutes of Health
SCAP	Apical Papilla	2006	W. Sonoyama, Y. Liu, D. Fang, T. Yamaza, B.M. Seo, C. Zhang, H. Liu, S. Gronthos, C.Y. Wang, S. Wang, S. Shi	USA. Los Angeles, California JAPAN. Okayama	University of Southern California School of Dentistry; Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences
DFSCs	Dental Follicle	2005	C. Morsczeck, W. Götz, J. Schierholz, F. Zeilhofer, U. Kühn, C. Möhl, C. Sippel, K.H. Hoffmann	GERMANY. Bonn	Stiftung Caesar, Center of Advanced European Studies and Research
hPCy-MSCs	human Periapical Cyst	2013	M. Marrelli, F. Paduano, M. Tatullo	ITALY. Crotone	Calabrodental, Unit of Maxillofacial Surgery; Tecnologica Research Institute, Biomedical Section

The periodontal ligament is another adult MSCs source in dental tissue, and periodontal ligament stem cells (PDLSCs) were isolated from extracted teeth [48]. PDLSCs have the ability to regenerate periodontal tissues such as the cementum, periodontal ligament and alveolar bone [49]. Moreover, MSCs have been also isolated from developing dental tissues such as the dental follicle (DFPCs)[50] and apical papilla (SCAPs) [51]. DFPCs have the ability to regenerate periodontal tissues whereas SCAPs demonstrate better proliferation and better regeneration of the dentin matrix when transplanted in immunocompromised mice with compared to DPSCs [50,52,53]. Zhang *et al.* have isolated mesenchymal stem cells from the gingiva, these MSCs exhibited higher clonogenicity, self-renewal and multipotent differentiation capacity similar to that of BMSCs [54]. Moreover, the salivary glands derived MSCs could differentiate into the salivary gland duct cells as well as mucin and amylase producing acinar cells *in vitro* [55]. In addition, De Bari *et al.* demonstrated that single-cell-derived clonal populations of adult human periosteal cells possess mesenchymal multipotency, as they differentiate to osteoblast, chondrocyte, adipocyte and skeletal myocyte lineages *in vitro* and *in vivo*. Therefore, expanded MSCs isolated from periosteum could be useful for functional tissue engineering, especially for bone regeneration [56].

The MSCs contained within the bone marrow aspiration from the iliac crest, and liposuction from extra-oral tissue are not easily-accessible stem cells. On the contrary, the orofacial bone marrow, periosteum, salivary glands and dental tissues are the most accessible stem cell sources. Moreover, the isolation of MSCs from these sources may still not be convenient because it requires surgical methods or tooth or pulp extraction. In addition, even if impacted wisdom teeth could be a mesenchymal stem cell source, these MSCs are present in a low percentage and can, therefore, be difficult to isolate, purify and expand. Furthermore, not all adults need the extraction of the wisdom teeth. To overcome these limitations, recently, Marrelli *et al.* demonstrated that MSCs derived from periapical cysts (hPCy-MSCs) have a mesenchymal stem cell immunophenotype and the ability to differentiate into osteogenic and adipogenic lineages [57]. The periapical cyst, which is a tissue that is easily obtainable and whose cells can be simply expanded from patients with minimal discomfort, seems to be a promising source of adult stem cells in dentistry for regenerative medicine. In fact, a recent study of Marrelli *et al.* showed that hPCy-MSCs similarly to DPSCs have neural progenitor-like properties by expressing spontaneously neuron and astrocyte specific proteins and neural related genes before any differentiation. Furthermore, hPCy-MSCs, under appropriate neural stimulation, acquire neural morphology and significantly over-express several neural markers at both protein and transcriptional level (in press, not yet published research by Marrelli *et al.*).

Mesenchymal stem cells in regenerative medicine

It was reported that MSCs isolated from whole bone marrow aspirates in combination with scaffolds and growth factors are able to repair cranial defects in several animal models [58-60]. These studies demonstrated that MSCs can alleviate the complications of craniofacial surgical procedures that required allogenic tissue grafts or extraction of autologous bone from secondary sites. This approach may alleviate donor site morbidity and allow a virtual unlimited source of cellular material derived from allogenic MSCs [61].

The identification of MSC residing in the oral cavity tissues increases clinical interest in MSCs as a cell source for regeneration of other connective tissues such as cementum, dentin and periodontal ligament (PDL). Many research studies research have been performed to assess the capacity of dental derived MSCs to enhance periodontal regeneration. Seo *et al.* have demonstrated that human PDLSCs were able to generate a cementum/PDL-like structures when transplanted into immunocompromised mice, and consequently transplantation of PDLSCs could be considered as a therapeutic approach for regeneration of tissues damaged by periodontal diseases [48]. Moreover, Kim *et al.* compared the alveolar bone regeneration achieved from implantation of PDLSCs and BMSCs and identified no significant difference in regenerative potential *in vivo* between these MSCs [62].

The three key elements in the field of tissue engineering are stem cells, scaffolds and growth factors [63]. Recently, researchers are trying to identify the ideal scaffold that facilitate growth, cell spreading, adhesion, integration and differentiation of MSCs. This scaffold should be biocompatible and biodegradable, should have optimal physical features and mechanical properties [64]. Different material have been designed and constructed for tissue engineering approaches, using natural or synthetic polymers or inorganic materials, which have been fabricated into porous scaffolds, nanofibrous material, hydrogels and microparticles. Natural materials include collagen, elastin, fibrin, silk, chitosan and glycosaminoglycans [65]. Recently, hydrogels have been investigated for tissue engineering applications because they offer numerous properties including biocompatibility and mechanical characteristics similar to those of native tissue [66,67]. Synthetic poly lactic-co-glycolic acid (PLGA) and titanium provide excellent chemical and mechanical

properties for bone tissue regeneration *in vivo* using DPSCs [68]. Furthermore, recent studies demonstrated that DPSCs loaded onto scaffolds of chitosan formed a dentine-pulp complex *in vivo* [69] whereas DPSCs cultured on hydroxyapatite (HA) and placed subcutaneously in nude mice formed bone [70]. A great number of investigations for evaluating the *in vivo* application of MSCs isolated from the oral cavity were carried out on animal models. A clinical study conducted by Papaccio's group gave evidence of the possibility to utilise DPSCs to repair bone defect in humans. In fact, they showed that DPSCs/collagen biocomplex completely restored human mandible bone defects subsequent to DPSCs transplantation [71].

Conclusions

The future is the regeneration of whole organs and complex biological systems consisting of many different tissues, starting from an initial stem cell line, probably using innovative scaffolds together with the nano-engineering of biological tissues: this approach is already a research topic in several international research institutes, and the best way to merge the numerous skills needed to get a so ambitious result is the multicenter collaboration. The authors are closely collaborating together with high-level international Universities, to develop protocols aimed to control and lead the tissues regeneration. This goal could make born a new generation of stem-cells based therapies, so to open the door to a new highly-performing regenerative medicine.

Starting from 2000, in only fifteen years, researchers have changed the face of the tissues engineering and the expectation of quality of life in more than 2 billions of patients undergone to a regenerative surgery: the challenge is to continue to make the patient's life better, to make the surgery more predictable and to simply replace damaged or degenerated tissues with MSCs from dental and oral sources.

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Competing Interests

The authors have declared that no competing interest exists.

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