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Bone Repair Cells for Craniofacial Regeneration

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Abstract

Reconstruction of complex craniofacial deformities is a clinical challenge in situations of injury, congenital defects or disease. The use of cell-based therapies represents one of the most advanced methods for enhancing the regenerative response for craniofacial wound healing. Both Somatic and Stem Cells have been adopted in the treatment of complex osseous defects and advances have been made in finding the most adequate scaffold for the delivery of cell therapies in human regenerative medicine. As an example of such approaches for clinical application for craniofacial regeneration, Ixmyelocel-T or bone repair cells are a source of bone marrow derived stem and progenitor cells. They are produced through the use of single pass perfusion bioreactors for CD90+ mesenchymal stem cells and CD14+ monocyte/macrophage progenitor cells. The application of ixmyelocel-T has shown potential in the regeneration of muscular, vascular, nervous and osseous tissue. The purpose of this manuscript is to highlight cell therapies used to repair bony and soft tissue defects in the oral and craniofacial complex. The field at this point remains at an early stage, however this review will provide insights into the progress being made using cell therapies for eventual development into clinical practice.

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2. Introduction

The craniofacial region is essentially composed of a framework of bone and cartilage giving support to muscles, ligaments, glands, and various layers of skin and subcutaneous structures. All these elements are innervated by the body's most sophisticated neurovascular network, which allows for function and sensorial capacities [1]. Injuries caused by trauma, tumor or cyst resection, infectious diseases, and also congenital and developmental conditions (i.e., cleft palate defects) may result into serious functional, aesthetical and psychological sequelae [2, 3]. In such situations, absence of hard and soft tissues can be disfiguring and often compromise basic functions such as mastication, speech, swallowing, and also lead to limited thermal and physical protection of important anatomical structures (i.e. brain, nerves, arteries, veins) [4–7]. The progression of certain oral conditions may also result in craniofacial defects of difficult resolution. Periodontitis is a chronic inflammatory disease of bacterial etiology, characterized by the loss of support around teeth, including alveolar bone resorption and soft tissue alterations [8–10]. Dental implant tooth replacements, one of the most popular therapies for total or partial edentulism, may be affected by a similar condition known as peri-implantitis [11]. Achieving predictable regeneration in the treatment of craniofacial defects is remarkably challenging in most clinical scenarios (Figure 1), given the loss of structural support and different embryologic origins of the affected tissues, among other factors.

Autogenous tissues have been widely used and are still considered as the *gold standard* to which all other biomaterials are compared [12]. Nevertheless, even the most advanced reconstructive techniques using autologous materials are often insufficient to restore extensive or complex maxillofacial defects [1]. Autografts contain all of the basic elements necessary to induce effective tissue regeneration, provided cells, extracellular matrix and cytokines [13, 14]. However, the use of autogenous tissue involves the need of harvesting it from a donor site, with the consequent drawbacks in terms of costs, procedure time, patient discomfort and possible complications. Additionally, oftentimes the volume of harvested tissues is not sufficient to fill or cover a defect, given the limited availability of autogenous tissues [15, 16]. To overcome these limitations, a variety of exogenous substitute materials, including allografts, xenografts and alloplasts, have been introduced in clinical practice over the last three decades [17, 18]. These materials primarily act as scaffolds, supporting the migration of cells from the periphery of the grafted area. Substitutes are indicated in the treatment of cases where the application of autografts alone may not be possible [19]. Unfortunately, when comparing these biomaterials to autografts other limitations emerge. The presence of *cellular populations*, orchestrate the release of *growth factors*, maintenance of a stable *scaffold*, and stimulate angiogenesis and are key for successful tissue regeneration as they play a fundamental role on the healing process [20]. Controlling the dynamics of these elements allows for a more predictable treatment of challenging craniofacial defects.

Novel tissue engineering therapies aimed at enabling clinicians to achieve predictable regeneration have been recently developed. These include, but are not limited to, the delivery of growth factors incorporated in carriers, the stimulation of the selective production of growth factors using gene therapy, and the delivery of expanded cellular constructs [21–67]. Approaches utilizing this latter strategy are known under the general name of *cell therapy*.

The incorporation of agents with biological properties into scaffolding materials has been proposed to modulate the behavior of precursor cells, that would ultimately contribute to the formation of new tissue [68, 69]. These agents can be grouped into two main categories: *growth factors and morphogens*. Growth factors primarily have mitogenic and chemotactic properties, while morphogens act through the alteration of cellular phenotype [70, 71]. Bone morphogenic proteins (BMPs) are an example of morphogens; they have the ability of inducing the differentiation of stem cells into bone forming cells in a process known as *osteoinduction* [72]. Other growth factors used in craniofacial regeneration include platelet-derived growth factor (rh PDGF-BB), transforming growth factor-beta 1 (TGF-β1), insulin-like growth factor-1 (IGF-1), vascular endothelial growth factor (VEGF), endothelial cell growth factor (ECGF), and fibroblast growth factor-2 (FGF-2). Although the application of these mediators has shown promising results in preclinical and clinical studies, suboptimal tissue response might occur as a consequence of the short half-life these molecules exhibit *in vivo* due to proteolytic degradation, rapid diffusion, or inadequate solubility of the carrier within the treated lesion [73].

In order to address growth factor delivery issues, the induction of a sustained release of growth factors via gene therapy was proposed [74–76]. *Gene therapy* basically consists of the insertion of genes into cells of the host, either directly or indirectly. This strategy was originally aimed at supplementing a defective mutant allele with a functional one in a therapeutic approach for some congenital conditions, but it can also be used to induce a more favorable host response [77, 78]. Targeting cells for gene therapy requires the use of vectors or direct delivery methods to transfect them [20]. In craniofacial regeneration, tissue engineering using gene therapeutic approaches may offer potential for optimizing the release of growth-promoting molecules, such as BMPs, in osseous defects [73, 79]. Although this approach is *per se* unique and relies on host cells for the new tissue production, several concerns regarding its safety have arisen [80, 81].

Another branch of tissue engineering has adopted the use of transplanted cells in order to promote and direct wound healing (Figure 2). *Cell therapy* approaches provide an additional source of cells in the area of interest, with the intent to be used as grafted cells (which will integrate into the patients body) or, when not intended for integration, as a source of growth factors [82, 83]. Cell therapy has a great potential in the clinical arena for the regeneration of both hard and soft tissues and could represent a new important instrument to enhance wound healing in different scenarios.

The purpose of this review is to examine the existing literature on the treatment of craniofacial defects adopting cell therapy approaches, to assess the validity of the different strategies, and to propose a path that can be shared by the research community toward which to direct future research efforts.

3. Cell Therapy Applications for Craniofacial Regeneration

Both somatic and stem cells can be used in cell based therapy (Table 1). Somatic cells can be harvested, cultured and implanted with the aim of engineering new tissues. Limitations in their use are related to the lack of self-renewal capability and limited potency; characteristics that are exclusive of stem cells [84]. Somatic cell delivery and stem cell therapy cells have been evaluated in different areas of regenerative medicine; their adoption in craniofacial regeneration will be discussed in the following paragraphs. Moreover, a recently developed cellular approach making use of both cell and gene therapies for the production of induced pluripotent stem (iPS) cells will be highlighted.

Somatic Cells

In the craniofacial region, fibroblast-like cells derived from the periodontal ligament have been used to promote periodontal regeneration [44, 45]. As demonstrated through in vivo investigations using a labeling technique, oral-derived periodontal cells are able to stimulate alveolar bone formation [46]. Cloned tooth-lining cementoblasts, periodontal ligament fibroblasts, and dental follicle cells seeded onto three-dimensional polylactic-co-glycolic acid scaffolds, exhibit mineral formation *in vitro* [47]. Immortalized cementoblasts delivered to large periodontal defects via biodegradable PLGA polymer sponges contributed to complete bone bridging and PDL formation, while dental follicle cells inhibited bone formation [48]. Another study showed that skin fibroblasts transduced by the BMP-7 gene promoted the regeneration of periodontal defects including new bone, functional PDL and tooth root cementum [49].

In the management of soft tissue defects cultivated fibroblasts have also been used for the treatment of interdental papillary insufficiency [52]. A human oral mucosa equivalent, made of autogenous keratinocytes on a cadaveric dermal carrier (Alloderm®) was able to favor wound healing when compared to the dermal carrier alone [53]. An *ex vivo* synthesized oral mucosa equivalent (EVPOME) produced without using animal-derived serum or feeder layer cells [54, 85] has demonstrated its ability to promote early initiation of epithelialization, short healing period and minimal scar contraction. This can be partially explained by the ability of this living construct to secrete growth factors as VEGF, promoting initial vascularization, which is critical to subsequent graft survival [86, 87]. EVPOME has been successfully used to treat patients affected by squamous cell carcinoma of the tongue, leukoplakia of the tongue, gingiva, and buccal mucosa or hypoplasia of the alveolar ridge [54]. In other soft tissue applications, allogenic foreskin fibroblasts have been utilized to promote keratinized tissue formation at mucogingival defects [50]. A tissue-engineered living cellular construct comprised of viable neonatal keratinocytes and fibroblasts rendered similar clinical outcomes when compared to conventional gingival autografts [51]. This construct has a strong potential to promote tissue neogenesis through the stimulation of angiogenic signals [88]. Another interesting product consists of the application of neonatal keratinocytes and fibroblasts for increasing keratinized gingiva around teeth [89]. This cell construct can stimulate the expression of angiogenic-related biomarkers as compared with autogenous free gingival grafts during early wound-healing stages [88] and, therefore, constitutes a promising material for gingival grafting without the need of a donor site.

The benefits of using somatic cells for the regeneration of soft and hard tissues in the craniofacial district have been illustrated by several preclinical and clinical studies [82]. Although, the lack of self-renewal capability and their commitment toward a single cellular phenotype limit their use in the treatment of more challenging craniofacial defects, in which a more orchestrated cellular response may be critical to gain success. Given their higher characteristics, stem cells might have a greater potential in this arena.

Stem cells

Stem cells retain the ability to perpetuate through mitotic cell division *(self-renewal)* and can differentiate into a variety of specialized cell types *(potency)*. The features of the tissue that will result from the regenerative process will be dictated by the cell-to-cell interaction at the defect site. For example, stem cell populations have the ability to differentiate into osteogenic cells as well as into ‘supportive osteogenic cells’, which may be of capital importance in the treatment of severely compromised bone defects. Supportive osteogenic cells are defined as cells that do not directly create bone, but that facilitate bone deposition by creating structures needed to allow this process (i.e., vascular network).

The bone marrow stroma contains hematopoietic stem cells and mesenchymal stem cells called Bone marrow stromal cells (BMSC) [90]. *Hematopoietic stem cells* give rise to blood cells of all lineages, while *Bone marrow stromal cells (BMSC)* are characterized by elevated renewal potency and by the ability of differentiating into osteoblasts, chondroblasts, adipocytes, myocytes and fibroblasts when transplanted *in vivo* [91]. From a single progenitor cell, limitative or inductive stimuli in the differentiation pathway may lead to cells characterized by lower renewal capacity and by an increased potential of differentiation [90]. This path seems to be reversible so that an adult adipocyte may de-differentiate back to levels with differentiation capabilities and then progress through the osteogenic pathway [92]. Studies have reported bone formation in ectopic places where MSCs were implanted [93] suggesting a possible role of MSCs in the production of different osteoinductive molecules [94].

MSCs can be obtained from a variety of sources [43, 95, 96]. Autologous MSCs isolated from a bone marrow aspirate from the iliac crest have been used to promote periodontal regeneration preclinically. The treatment allowed for the regeneration of cementum, periodontal ligament, and alveolar bone [30, 97]. Bone marrow mesenchymal stem cells seeded in an engineered porous poly-L-lactic acid - polyglycolic acid composite scaffold have been adopted to graft extraction sockets in an animal model resulting in better preservation of alveolar bone walls than in control groups [31]. Bone block allografts impregnated with bone marrow aspirated from the anterior iliac crest offered a predictable and a cost effective therapy for the treatment of severely atrophic maxillary and mandibular ridges when compared to harvesting autogenous bone [29].

Clinical studies have demonstrated excellent results when combining bone marrow aspirations and platelet-rich plasma (PRP), an autologous product that contains supraphysiologic levels of platelets, in alveolar ridge augmentation procedures [32]. A similar combination therapy was able to improve osseointegration of dental implants [33, 34], to enhance bone regeneration during implant site development techniques [35] and for the treatment of periodontal angular defects [36].

MSCs may also be harvested from adipose tissue with the advantage of a high ease of access with low morbidity, because of the large amounts of human lipoaspirates readily available [98, 99]. Adult adipocytes are able to de-differentiate back to levels with higher generative capabilities. Studies on rats suggested that adipose-derived stem cells mixed with PRP can promote periodontal tissue regeneration [37]. Following the positive results of tissue engineering strategies for the reconstruction of long bones and large osseous defects in orthopedic surgery [100–102], significant efforts were made in regenerating cartilage. Since adult chondrocytes are characterized by a limited proliferative potential, focal chondral lesions (that do not contain a vascular network) do not heal and osteochondral lesions (which receive partial vascularization from the osseous tissue) typically heal by formation of non-functional fibrous tissue [103]. An attempt to produce human articular chondrocytes *in vitro* has been successfully performed and the cultured cells have been tested in a knee-healing model [55–58]. Despite the encouraging results, chondrocyte culture conditions and graft fixation methods still present limitations. Given their high proliferation and differentiation potential, the adoption of stem or progenitor cells to treat cartilage defects could represent a promising approach and would not require resection of healthy cartilage tissues. Tissue engineered cartilage formed in bioreactors [104] and osteochondral composite tissues have been generated *in vitro* [105, 106] and successfully utilized *in vivo* [59, 107]. Repair of osteochondral defects at high load-bearing sites in adult rabbits was achieved by using PLCL-based sponge scaffolds and BMSCs [38]. Recently, the use of a composite material consisting of NELL-1 (NEL-like molecule-1)-modified autogenous bone marrow mesenchymal stem cells (BMMSCs) and poly lactic-co-glycolic acid (PLGA)

scaffolds has been tested in the treatment of surgically-created osteochondral defects in goats' mandibular condyles [39]. This approach could be potentially applied for the resolution of temporomandibular joint injuries, which is often challenging with conventional treatments.

Bone marrow, adipose tissue, liver and muscle are known sources of postnatal stem cells, but even intraoral sites, such as the dental pulp and the periodontal ligament, can be used as a source of MSCs [108–110]. Cells derived from various dental tissues were shown to maintain the ability to differentiate into osteoblasts, adipocytes, and other cell types [111]. Perry and collaborators suggested the possibility of banking MSCs through cryopreservation of extracted third molars, because cells recovered from the pulp maintained the characteristics of MSCs [112]. Similar characteristics were observed on cells isolated from deciduous dental pulp including the ability of generating dentin and dental pulp [113–115]. Also cells from human periodontal ligament were found to have MSCs features and were able to generate PDL-like structure *in vivo* [116]. Post-natal stem cells were also recovered from cryopreserved periodontal ligament of previously extracted teeth [117]. Periodontal regeneration was more robust when using autologous periodontal ligament cells obtained from extracted premolars and prepared in sheets using temperature-responsive cell culture dish technique and hyaluronic acid carrier [40]. PDL stem cells have also shown the potential to regenerate periodontal attachment apparatus in vivo in a porcine model including new bone, cementum and PDL [41]. PDL stem cells express several mesenchymal stem cell markers, such as STRO-1 and CD44, and maintain the ability to differentiate into osteogenic, adipogenic, and chondrogenic pathways [82, 118].

After extracting tooth germ progenitor cells from discarded third molars, Ikeda and co-workers suggested the possible use for regeneration of fatal disorders as for cell-based therapy to treat liver diseases [119]. Iohara *et al.* demonstrated reparative dentin formation in a dog model using BMP2-treated pellet culture of pulp progenitor/stem cells [42].

In vivo generation of a tissue-engineered natural tooth, including all of its supporting structures and capable of completely replace functionally and aesthetically its missing counterpart, is a current utopia. Thanks to advances in cell therapy materialization of that concept seems to be getting closer and closer. Sonoyama *et al.* were able to generate a "bio-root" structure encircled with PDL tissue by combining PDL stem cells with stem cells from the root apical papilla of human teeth [120]. Recently, cell transplantation of PDL progenitor cells, collected from extracted teeth, expanded in bioreactors and delivered in the surface of titanium implants, has shown the proof-of-principle to generate hybrid ligament-dental implant constructs [60]. For the first time in a case series of human participants it was possible to induce formation of a biological ligament at the interface between these "ligaplants" allowing them to withstand functional loading for extended periods of time [60, 121, 122].

As such, there is significant potential for the use of either stem cells or PDL progenitor cells to form both soft and hard periodontal tissues in vivo.

The latest advancement in stem cell therapy is related to the use of induced pluripotent stem (iPS) cells. These cells populations have the similar characteristics of embryonic stem cells in terms of cell morphology, proliferation, surface antigens, gene expression, telomerase activity, and epigenetic status of pluripotent cell specific genes [123–125]. The generation of iPS cells usually requires the combined adoption of cell and gene therapies. The use of retroviruses, lentiviruses, adenoviruses, plasmid transfection, transposons, and recombinant proteins are among the different strategies to produce iPS cells [126]. The potential of iPS cells is remarkable, as they might allow for the use of stem cells without the hassle and

possible complications of the surgical maneuvers needed for harvesting cells from the patient bone marrow.

A Japanese group reprogramed mouse somatic cells and adult human dermal fibroblasts to generate iPS cells [123, 124]. Both at the Genome Center of Wisconsin [125] at the Children's Hospital Boston and at the Dana Farber Cancer Institute [127] researchers were able to derive iPS cells from human somatic cells reprogram somatic cell nuclei to an undifferentiated state. Collaborations between Kyoto and Gifu Universities for the establishment of an iPS cell bank of various human leukocyte antigen (HLA) types generated 2 cell lines, which are estimated to cover approximately 20% of the Japanese population with a perfect match [128].

To date, several concerns have arisen related to the use of iPS cells in humans, including the limited efficiency of reprogramming primary human cells (making it difficult to generate patient-specific iPS cells from initially small cell populations), the possible integration of viral transgenes into the somatic genome, which may potentially induce tumorogenesis [129], and iPS cell teratoma formation [130–132]. Even a small number of undifferentiated cells can result in the formation of a teratoma. Therefore, regardless of the advances demonstrated thus far, the potential for tumor formation has not yet been eliminated [126] and the use of autologous cell sources remains the safest approach to Stem Cell Therapy.

Scaffolds for Cell Therapy Delivery to Oral and Craniofacial Defects

Scaffolds play a pivotal role in providing a three-dimensional template for tissue neogenesis [133]. Scaffolds can not only be used as carriers for cell delivery but they serve as synthetic extracellular-matrix environments to define a 3D geometry for tissue regeneration and provide an adequate microenvironment in term of chemical composition, physical structure and biologically functional moieties [134, 135]. Thus far, the most widely adopted scaffolds for craniofacial bone regeneration are xenogenic and allogenic bone substitutes, hydroxyapatite, calcium phosphates, and gelatin or collagenous sponges [30, 46, 62–64]. Limitations in their use are related to the lack of degradability of certain materials or too fast degradability of others, poor processability into porous structures, brittleness, inability to generate structures to be tailored to the specific needs of the patient or inability to maintain the desired volume under mechanical stimuli. In order to overcome these limitations synthetic scaffolds specifically designed to mimic the wound healing extracellular matrix are being evaluated.

This biomimetic concept applied to materials synthesis intends to generate biodegradable scaffolds with a highly porous structure and adequate mechanical properties for bone engineering [133]. Ideally, a scaffold material should be degradable at a rate similar to that of the new tissue formation, large interconnected pores are required to allow for cell incorporation, migration, and proliferation [136]. Bone formation occurs over a structured collagen matrix with fiber bundle diameter varying from 50 to 500 nm [137, 138], therefore nanofibrous scaffolds appear to provide better cellular attachment [139], increased differentiation of osteoblastic cells [140], and enhanced mineral deposition compared to solid-walled scaffolds [141].

Electrospinning, self-assembly, and phase separation are three different methods employed in the fabrication of nano-fibrous polymeric scaffolds for tissue engineering. Electrospinning is a simple method, which utilizes an electric field to draw a polymer solution from an orifice to a collector, producing polymer fibers [142, 143]. It can be used is used to produce thin two-dimensional sheets, while three-dimensional nanofibrous scaffolds have been fabricated by layering these 2D sheets [144] or by combining electrospinning with 3D printing [145]. Molecular self-assembly uses non-covalent bonds such as hydrogen bonds,

van der Waals interactions, electrostatic interactions, and hydrophobic interactions for fabricating supramolecular architectures [146]. Limitations in the use of self-assembly methods are related to difficulties in forming macropores and limited mechanical properties [147]. Finally, thermally induced phase separation (TIPS) technique can be used to fabricate nano-fibers through polymer dissolution, phase separation and gelation, solvent extraction, freezing, and freeze-drying under vacuum [148]. This technique can also be combined with processing techniques such as particulate leaching or 3D printing to design complex 3D structures with well-defined pore morphologies [140, 149, 150].

Another interesting aspect of polymer scaffolds is that CAD/CAM technologies can be applied to create patient-specific, anatomically shaped scaffolds. As craniofacial defects and anatomical stuctures may greatly vary among different individuals a scaffold unique to each patient can be helpful in regenerating defects with complex geometry [133].

Polymers have great design flexibility and their composition and structure can be designed to match the specific needs of the tissue to be engineered. Moreover, benefits can be reached by adding nano-crystalline hydroxyapatite to the scaffolds as it has a strong potential for attracting osteoblasts (osteoconductivity), it improves its mechanical properties [151], and may reduce adverse effects associated with the degradation of some synthetic polymers [147]. Hydroxyapatite crystals can be incorporatied during processing of polymer scaffolds or they could be biomimetically grown onto a prefabricated polymer scaffold. Since all interactions with biological components occur at the pore surface, the non-exposed ceramic is in effect wasted [147] and could affect biodegradability and mechanical properties of the scaffold. It is therefore recommended to allow apatite to form as a coating of the polymer scaffold in order to enhance its surface characteristics. An interesting technology has been described in which prefabricated polymer scaffold are soaked in simulated body fluid in order to allow apatite crystals to grow onto its pore surfaces [152, 153].

Growth factors can easily be incorporated in polymeric scaffolds [154–156], which would allow for a more sustained release of the molecules and better properly orchestrated tissue formation. As such, 3D porous, nanofibrous scaffolds have supported various stem cells and differentiated cells to regenerate many hard and soft tissues. It should be pointed out that significant technical challenges remain for the synergistic integration of structural cues with biological cues for cell-based therapies to achieve functional dental and craniofacial tissue regeneration [157]. However, it is likely that the continuous expansion of biomimetic approaches in the scaffolding materials design will substantially advance the field of tissue engineering and regenerative medicine. Recently, a biomimetic fiber-guiding scaffold using solid free-form fabrication methods that custom fit complex anatomical defects to guide functionally-oriented ligamentous fibers in periodontal regeneration has been successfully tested *in vivo* [158] and work is being done to incorporate biomimetic scaffolds in cellular delivery for craniofacial bone regeneration in many other clinical scenarios (Table 2).

4. Bone Repair Cells (Ixmyelocel-T)

Bone repair cells (generic term Ixmyelocel-T) are a patient-specific multicellular therapy manufactured from a small sample of autologous bone marrow marrow in a proprietary, closed-system bioreactor. Ixmyelocel-T has been evaluated both clinically and preclinically, in multiple applications in cardiovascular, neurological and orthopedic surgery [159–166]. For example, a recent case report showed successful results in regenerative facial reconstruction of terminal stage osteoradionecrosis and other serious craniofacial conditions [167]. This expanded mixture of cells contains MSCs with bone-forming potential in preclinical animal models [168], however this mixture has not yet been fully-optimized for bone regeneration applications, and in fact is currently in clinical trials for critical limb

ischemia and dilated cardiomyopathy. In these models ectopic bone formation has not been reported widely using many different bone marrow derived cells and MSCs from various sources. Thus the impact of the heterogeneous cell populations specifically on bone formation remains to be fully understood.

When using a simple autologous bone marrow aspiration for a bone grafting procedure some of the cells populating bone marrow have strong osteogenic capacity, while others have essentially no intrinsic bone-forming potential (eg. monocytes and macrophages) though they may regulate indirectly or in a paracrine manner [169], though this is primarily hypothetical at this stage. Culturing protocols, therefore, should be aimed at expanding those cells with osteogenic capacity while reducing inhibiting cells. The characterization of ixmyelocel-T cell populations has been previously described [168] and we previously reported on the phenotypic characterization of ixmyelocel-T samples from patients treated in a recently conducted clinical study [64]. Significant to note was the finding that these cell populations were highly enriched for CD90 and CD105 positive cells. CD105 was originally identified as a marker of mesenchymal stem cells [170], and subsequently associated with vascular endothelia in angiogenic tissues [171]. CD90 is expressed by bone marrow subpopulations of colony-forming mesenchymal stem cells (CFU-F, colony-forming unit–fibroblasts) [172]. It was also demonstrated that Ixmyelocel-T populations produced significant concentrations of angiogenic cytokines and showed the ability to differentiate into endothelial cells [64]. The therapeutic implications of these findings are that Ixmyelocel-T may not only serve to provide a source of stem and progenitor cells to a wound healing site, but may also be actively involved in the establishment of a supportive, vasculature which can support and sustain tissue regeneration.

Our group recently completed a Phase I/II, proof-of-concept, feasibility study where we randomly assigned 24 subjects to either a control group (Guided Bone Regeneration [GBR] with gelatin carrier alone) or to a test group (cell therapy with Ixmyelocel-T adsorbed into the gelatin sponge with GBR). For more information, see www.clinicaltrials.gov: NCT00755911. After either 6 or 12 weeks of healing, bone core biopsies were harvested and dental implants were installed. The test groups allowed more bone formation with lower degree of ridge resorption. Bone density was measured by tactile means during clinical assessment, micro-computed tomography (micro-CT) and, additionally, histological analyses were carried out. Bone regenerated with this cell therapy was found to be of higher density than bone regenerated using GBR. Histological evaluation of the biopsy specimens revealed formation of highly vascular, mature bone as early as 6 weeks after implantation.. Our study demonstrated that cell therapy for regeneration of alveolar bone defects is safe and accelerates the early stages of osteogenesis. It also establishes preliminary evidence for consideration of larger scale clinical studies for the use of Ixmyelocel-T therapy in the treatment of more complex craniofacial defects [63, 64]. In addition to this pilot study, our group is conducting an ongoing Phase I/II placebo controlled, randomized human clinical trial investigating the potential of Ixmyelocel-T to stimulate bone formation in severely resorbed alveolar ridges in the maxillary arch. In this investigation (www.clinicaltrials.gov: NCT00980278), patients requiring maxillary sinus floor augmentation and dental implant placement are randomized to receive beta tricalcium phosphate (β-TCP) bone filler as a control, standard-of-care therapy, or β-TCP loaded with Ixmyelocel-T. The patients will be followed over a one-year period and total bone volume and oral implant success will be assessed.

Important considerations to utilization of ixmyelocel-T as a cell therapy are the cost, the need to harvest a bone marrow sample from the iliac crest, and the time required to expand the cells (12–14 days) prior to their use. The regenerative potential of ixmyelocel-T may also be affected by the biomaterial used as a carrier to deliver the cells to the regenerative

site. In general, when osseous defects are localized and well-contained, as in the case of a tooth extraction socket, a non-mineralized carrier (ie. gelatin sponge) may be more appropriate to use in that it has good handling properties and easily conforms to the defect site. However, many larger more complex defects that could benefit from ixmyelocel-T treatment are not self-contained. It is these clinical situations, often requiring vertical and horizontal osseous augmentation, that are among the most difficult to treat in that the structural integrity of the graft is paramount in providing the maintenance of the space required for regeneration of the tissue [173]. Generally, mineralized blocks or mineralized particulate bone grafts in combination with resorbable membranes or titanium-reinforced ePTFE membranes are used for the treatment of these types of defects. However, the recent emergence of 3D polymer scaffolding technologies, could represent an optimal solution for the delivery of ixmyelocel-T, once technical limitations are overcome [133, 147, 157].

5. Expert's Outlook

Advances in tissue engineering open the possibility of utilizing new therapeutic protocols for the treatment of large osseous defects in the craniofacial area including the cranium, jaws and localized periodontal deformities. Bioengineering strategies using stem cells may allow predictable therapeutic approaches with the potential of reducing the limitations of current state-of-the-art clinical protocols. To date, the use of cell therapies for oral craniofacial regeneration is quite limited and reserved to orphan product status for most indications. Some of the first cell therapies receiving FDA approval are limited to the use of neonatal fibroblasts/keratinocytes that received FDA panel review (NCT00587834), but awaiting FDA full approval for oral application. For bone regeneration, the use of cell therapies have many of the practical challenges of harvest, procurement and expansion via bioreactors. The steps involved make the regenerative therapies more expensive and time-consuming as compared to the use of growth factors that have received approval in the craniofacial complex such as rhPDGF-BB or BMP-2 [174]. However, given some of the limitations of protein-based therapies in providing predictable bone regeneration (in terms of consistency of result and extent of bone volume), cell therapies indeed offer a viable alternative to protein-based growth factors and allograft tissue transplants. Clinical trials ongoing using Ixmeyelocel-T in alveolar ridge (NCT00755911) and sinus floor augmentation (NCT00980278) offer potential for the use of stem cells for these application, however these studies are at the Phase 1/2 stage and will require further validation in larger, multi-center investigations.

At this point in time our expert outlook is that cell therapies will have a place in regenerative medicine, and in particular will be most highly used in the treatment of advanced defects in facial reconstruction. These cellular therapies lend themselves to delivery using image-based, CAD approaches to repair major craniofacial defects of complex morphologies where cells will have the unique potential to form into multiple tissues to address the complex form and function required in the oral and craniofacial complex.

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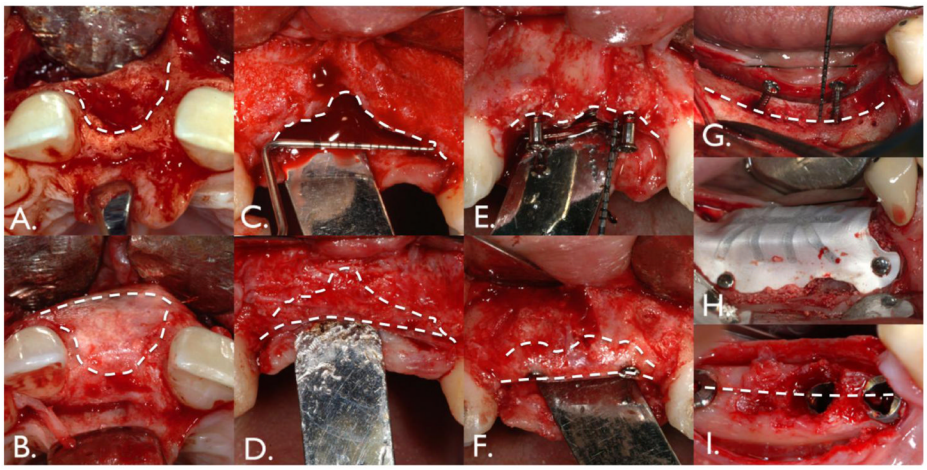

Figure 1. Tissue engineering applications in the craniofacial complex

Several clinical scenarios can benefit from the application of tissue engineering approaches, such as cell therapy. In order to accommodate dental implants deficient maxillary and mandibular alveolar ridges can be expanded horizontally **(A, B)**, vertically **(C, D)**, or both vertically and horizontally **(E, F, G, H)**.

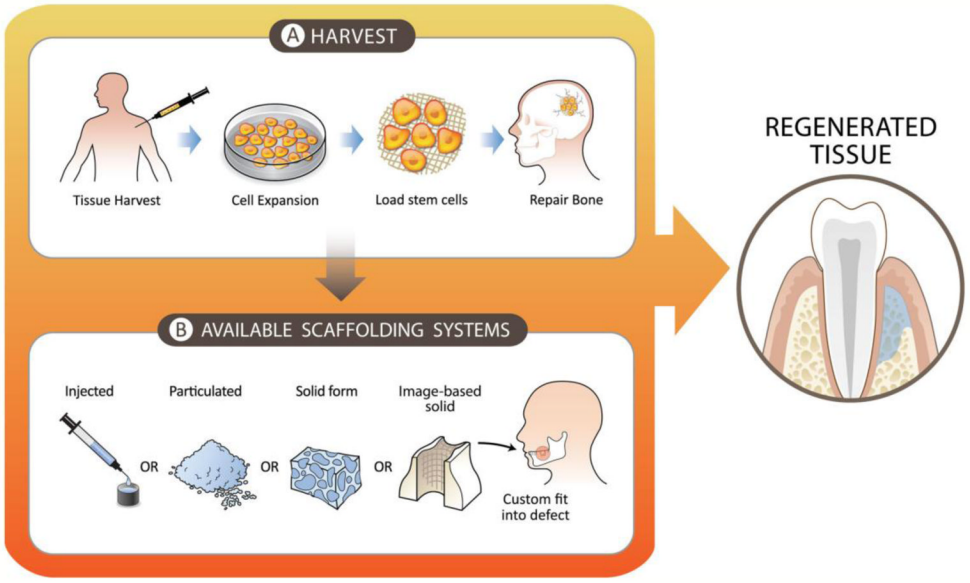

Figure 2. Cell therapy technologies for regenerative medicine

A) Cell therapy provides an additional source of cells in the area of interest. After harvesting a tissue sample, the cells are expanded, manipulated, and loaded onto a carrier. Similarly, Ixmyelocel-T is harvested from the own patient, expanded through a completely automated and closed SPP system and loaded into a scaffolding material (i.e. gelatin foam, β-TCP). When grafted in a bone defect, Ixmyelocel-T promotes enhanced bone regeneration and maturation.

B) Injected, particulated, prefabricated solid, or image-based solid scaffolds are available in tissue engineering. Thanks to the integration of these newly available technologies new perspectives for enhanced outcomes in the regeneration of craniofacial structures can be explored.

Table 1

Cell therapy applications for periodontal/craniofacial tissue engineering.

	Regenerative cell construct	Study model	References
Autologous Stem Cells	Bone block allografts impregnated with autogenous bone marrow	Patients with severely atrophic maxillary and mandibular ridges	Soltan *et al.* 2007 [29]
	Autologous MSCs isolated from a bone marrow aspirate and expanded *in vitro*	Periodontal regeneration in class III furcations in a dog model	Kawaguchi *et al.* 2004 [30]
	Engineered porous scaffold seeded with BMSCs	Postextraction socket in rabbits	Marei *et al.*, 2005 [31]
	PRP + MNCs from bone marrow aspirate	Alveolar ridge augmentation in humans	Filho Cerruti *et al.,* 2007 [32]
	PRP + *in vitro*-expanded bone marrow derived MSCs	Trephined defects in dog mandibles	Yamada *et al.*, 2004 a,b,c [33–35]
	PRP + *in vitro*-expanded bone marrow derived MSCs	Periodontal defects in humans	Yamada *et al.*, 2006 [36]
	Adipose-derived stem cells	Periodontal defects in Wistar rats	Tobita *et al.*, 2007 [37]
	BMSCs incorporated with a PLCL scaffold	Osteochondral defect on the medial femoral condyles at a high load-bearing site on a rabbit's knee joint	Xie *et al.,* 2010 [38]
	NELL-1 modified autogenous BMSCs in PLGA scaffold	Surgically-created osteochondral defects in goats' mandibular condyles	Zhu *et al.*, 2011 [39]
	Autologous periodontal ligament cells from extracted teeth in a hyaluronic acid carrier	Dehiscence defects in beagle dogs	Akizuki *et al.*, 2005 [40]
	PDL stem cells from extracted teeth	Surgically-created periodontal defects in miniature pigs	Liu *et al.*, 2008 [41]
	Bmp2-supplemented dental pulp stem cells	On amputated pulp to stimulate reparative dentin formation	Iohara *et al.* 2006 [42]
	BMSCs cryopreserved for 1 month and freshly isolated BMSCs (control)	Periodontal fenestration on beagle dogs	Li *et al.* 2009 [43]
Allogenic Somatic Cells	Fibroblast-like cells from expanded regenerated periodontal ligament cells	Artificial class II furcal defect in a dog model	Dogan *et al.*, 2002, 2003 [44, 45]
	Periodontal ligament cells	Periodontal defects created in Sprague-Dawley male rats	Lekic *et al.*, 2001 [46]
	Cultured cementoblasts, periodontal ligament fibroblasts, and dental follicle cells	Ectopic tissue regeneration in mice using 3-D poly lactic-co-glycolic acid (PLGA) scaffolds	Jin *et al.*, 2003 [47]
	Cultured primary follicle cells and immortalized cementoblasts	Buccal periodontal defects in mandibular molarf of athymic rats	Zhao *et al.*, 2004 [48]
	Syngeneic skin fibroblasts transduced by the BMP-7 gene	Periodontal ligament regeneration at sites with periodontal bone defects in rats	Jin *et al.*, 2003[49]
	Living human fibroblast-derived dermal substitute (Allogenic foreskin fibroblasts and keratinocytes)	Patients with insufficient attached gingiva	McGuire *et al.*, 2005 [50]
	Living human fibroblast-derived dermal substitute (Allogenic foreskin fibroblasts and keratinocytes)	Multi center study treating patients with insufficient attached gingiva but no need for root coverage	McGuire *et al.*, 2007 [51]
Autologous Somatic Cells	Periodontal ligament cell sheets with reinforced hyaluronic acid carrier	Surgically create dehiscence defects	Akizuki *et al.*, 2005 [40]

	Regenerative cell construct	Study model	References
	Cultured and expanded autologous fibroblasts	Injections for papilla priming procedure to augment open interproximal spaces	McGuire *et al.*, 2007 [52]
	Ex vivo produced oral mucosa equivalent (EVPOME, Autogenous keratinocytes seeded on Alloderm®)	Patients with either a premalignant or cancerous mucosal oral lesion	Izumi *et al.*, 2003[53]
	Ex vivo produced oral mucosa equivalent (EVPOME, Autogenous keratinocytes seeded on Alloderm®	Patients affected by squamous cell carcinoma of the tongue, leukoplakia of the tongue, gingiva, and buccal mucosa or hypoplasia in the alveolar ridge	Hotta *et al.*, 2007 [54]
	Autogenous chondrocytes expanded in presence of FGF-2 and TGFβ1	Cartilage defects in the knee	Brittberg *et al.*, 1994, Jakob *et al.*, 2001, Dozin *et al.*, 2002, 2005[55–58]
	Engineered cartilage generated in vitro from chondrocytes cultured on a biodegradable scaffold	Osteochondral defect in a rabbit knee joint	Schafer *et al.,* 2002 [59]
	PDL-derived cells cultured and placed on the surface of Ti pins	Implantation on nude mice, beagle dogs and human patients	Gault *et al.,* 2010 [60]

Table 2

Scaffolding Matrices for Delivery of Cells for Craniofacial Tissue Engineering.

	Biomaterial	**Scaffold**	**Cell Therapy**
Naturally Delivered	Allografts	Bone block allografts	Extraoral MSCs (Bone marrow MSC) [29]
	Xenografts	Collagen sponge	Oral/craniofacial MSCs (Pulp cells) [61]
		Gel/Gelatin	Oral/craniofacial MSCs (PDL cells) [46]
			Extraoral MSCs (Bone marrow MSC) [30]
			Oral/craniofacial MSCs (PDL cells) [62]
			Extraoral Expanded Stem Cells (BRCs) [63, 64]
Synthetic/Alloplasts	Polymers	PLLA (polylactic acid)	Oral MSCs (PDL fibroblasts) [65]
		PLGA (poly[lactide-co-glycolide]) (co-polymer of PLLA & PGA)	Oral/craniofacial MSCs (cementoblasts) [47]
			Extraoral MSCs (Bone marrow MSC) [31]
	Ca-P based ceramics	Tricalcium phosphate (β-TCP), calcium phosphate cement	Extraoral Expanded Stem Cells (BRCs)
	Hydroxyapaptite-based scaffolds	Hydroxyapaptite dense HA, porous HA, resorbable HA, Non-porous non-resorbable granular HA	Oral/craniofacial MSCs (PDL cells) [60]
		Hyaluronic acid ester	Oral/craniofacial MSCs (PDL cells) [40]
		Porous HA	Expanded bone marrow MSCs [66]
		Hydroxyapaptite/ Tricalcium phosphate	Bone Marrow MSCs [67]