Cell Culture–Based Tissue Engineering as an Alternative to Bone Grafts in Implant Dentistry: A Literature Review

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Several biomaterials and techniques for bone grafting have been described in the literature for atresic bone tissue replacement caused by edentulism, surgical resectioning, and traumas. A new technique involves tissue engineering, a promising option to replace bone tissue and solve problems associated with morbidity of autogenous grafting. This literature review aims to describe tissue-engineering techniques using ex vivo cell culture as an alternative to repair bone maxillary atresias and discuss the concepts and potentials of bone regeneration through cell culture techniques as an option for restorative maxillofacial surgery.

Key Words: tissue engineering, bone transplantation, bone regeneration, stem cells

INTRODUCTION

More than 2.2 million bone graft procedures are performed annually in dentistry, neurosurgery, and orthopedics.[1] The popularity of dental implants has generated an even higher demand for dentoalveolar bone reconstruction.[2]

There are 4 functional principles that characterize the biology of biomaterials applied for bone grafting. These are osteointegration, which is the ability of the bone tissue to directly adhere to the surface of the implant biomaterial without forming a fibrous interface; osteoconduction, which is the ability to support and guide bone growth on the surface of the grafted material; osteoinduction, which is the capacity to induce mesenchymal stem cells to differentiate into osteoblasts in the region of interest; and osteogenesis, meaning the capacity to form new bone tissue through the cells present at the grafting material.[3,4]

One prerequisite for oral rehabilitation in osteointegration is the presence of a remnant alveolar process, which may allow the installation of dental implants aimed to withstand a functional prosthesis over a long period of time.[5,6] For this reason, various biomaterials have been tested and suggested as alternatives for bone reconstruction in the dental literature. These graft materials could be divided by their origin as autogenous, allogeneic, xenografts, or synthetic.[1,2,4]

Autogenous grafts have the best clinical response in terms of newly formed bone tissue because they come from the same individual and therefore present properties such as osteogenesis, osteoinduction, and osteointegration.[3] Osteoprogenitor cells present in the autogenous grafts may promote new bone tissue formation, as marrow elements may facilitate the combination with osteoinductive proteins and osteogenic cells and also help to establish local vascular support.[1,4,7,8] Despite a positive clinical response, autogenous grafts have limitations on their clinical use because of factors such as donor site morbidity and the amount of available material.[9,10] In addition, the literature has shown that 8% to 20% of surgeries involving autogenous grafts are associated with postoperative complications, including hematomas, blood loss, nerve and vessel injury, infections, bone

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fractures, and occasional chronic pain at the donor site.[11]

Allografts are usually taken from individuals of the same species but a different genotype from the host. The allogenous materials for grafting may undergo different manufacturing processes, such as physical debridement, ultrasonic washing, ethylene oxide treatment, antibiotic washing, and gamma radiation application, aiming mainly for decontamination before their clinical application. Because of these processes, many biological, mechanical, and osteoinductive material properties are lost or significantly reduced, thereby negatively affecting their clinical response.[1,7]

Xenografts present a genetically different origin than the hosts because of their frequent animal origin, and they are the grafts most often used in dentistry.[12] Also, synthetic materials such as bioactive glass[3] and bioceramic materials[1] are also available and widely applied in bone reconstructions, although they present less expressive clinical responses.[7]

Given the reported disadvantages in terms of clinical responses from using alternative biomaterials for bone grafting and also significant disadvantages associated with the use of autogenous bone grafts, the search for new reparative treatment alternatives has become significant in the past years. Among these alternatives, tissue-engineering techniques, involving autogenous cell cultures ex vivo combined with a specific matrix or scaffold, frequently associated with growth factors, have been described in the dental literature as promising alternatives for reconstructive bone surgery.[9,10]

The aim of this review is to investigate the dental literature regarding the current status and applicability of cell culture–based tissue engineering as a bone tissue reparative technique, investigating its potential as a viable clinical alternative to conventional bone grafts, and also detailing its possible application in maxillary reconstructions for implant dentistry.

Tissue engineering

The loss or malfunction of organs or tissues constitutes nowadays a common health problem. A new field of biomedical science, known as tissue engineering, applies the principles of biology and engineering to the development of functional substitutes for tissues and organs.[13]

Tissue engineering is also characterized as a new and exciting field that attempts to recreate functional tissues and organs lost in different ways. This new proposal demands multidisciplinary knowledge of biological sciences and has the potential to significantly affect medical and dental therapies. Based on the principles of cellular biology, many types of tissues can potentially be grown in vitro, such as bone tissue, oral mucosa, dentine and dental pulp, skin, and salivary glands.[14]

The term *tissue engineering* describes the production of organic tissues through techniques of cellular proliferation in vivo or ex vivo, which may be combined with a scaffolding material and/or growth factors. Many cell types, including cells with an osteoblast phenotype, can proliferate and maintain their phenotypes in culture, first on bidimensional substrates or in porous matrices and 3-dimensional gels in vitro.[13]

Commonly employed strategies for tissue engineering require a previous biopsy of live tissue, containing the cells of interest for the receptor site. Afterward, cell cultures are developed in the laboratory, where the cells of interest are expanded and seeded onto polymer matrices so that they may later be reinserted into the organism.[14] The triad that constitutes the base of tissue engineering with a reparative objective is formed by the following: matrices or scaffolds, with various presentations (gels, fibrous matrices, permeable membranes), progenitor cells (undifferentiated stem cells, or cells with preliminary differentiations), and growth factors.[15,16]

Mesenchymal stem cells

Currently, there is a great interest in developing techniques for the laboratory manipulation of stem cells present in organs and tissues to provide restorative clinical treatments for damaged organs and tissues.[17] Therapies using these cells for tissue repair and regeneration have a great potential as therapeutic solutions for a large number of clinical situations involving tissue damage and/or loss repair.[18]

The 2 large groups of stem cells with potential applications in tissue engineering are totipotent or pluripotent embryonic stem cells and the lineages of unipotent or multipotent adult stem cells found in differentiated tissues.[19] Embryonic stem cells found in the blastocyst and primordial germ cells

possess high plasticity; therefore, these cells present a great potential for differentiation as they are able to give rise to all types of cells and consequently tissues and organs.[20] Also, adult stem cells, such as mesenchymal cells, are commonly found in the bone marrow and conjunctive tissues. They can produce certain cell lines, such as osteoblasts, chondroblasts, adipocytes, tenocytes, myoblasts, and stromal cells.[21]

Although embryonic stem cells can generate a large variety of cell lines and theoretically have great clinical potential for tissue engineering, there are currently many limitations to their use in clinical and research applications. These limitations are due to legal regulatory policies and ethical considerations regarding the use of these cells, which may vary considerably depending on the legislation of each country or region.[18]

Given the limiting factors of these more primitive cell lines, the use of adult stem cells in tissue engineering in dentistry has gained strength because of the availability and acceptance for clinical use to regenerate and/or reconstruct bone, pulp, and periodontal tissues.[22] For these purposes, adult stem cells are more readily available and do not raise significant ethical or immunoreactive issues.[18]

Although there may be many advantages, the collection of autogenous MSCs from tissues, such as the bone marrow or adipose tissue, might be associated with minor morbidity at the donor site. However, original adult stem cells from adipose tissue are capable of differentiating into mesodermal cells and tissues. Thus, the collection of a large quantity of these cells from a patient under local anesthesia constitutes a viable clinical alternative,[18] as these cells present a high differentiation potential necessary for cell therapies in tissue engineering.

In addition to the previously described qualities, mesenchymal stem cells are easily isolated and cultured in vitro. These cells possess a high proliferative potential, are easily stored in freezing conditions without altering their osteogenic potential, and adhere easily to plastic surfaces.[23] Osteoblastic characteristics of these cells may be directly linked to the presence of bone morphogenic proteins of the transforming growth factor beta (TGF-β) family in their culture environment and also to the amount of viable cells that can trigger bone growth, such as in the process of embryological development.[24] When cultivated in vitro, they may be previously placed in a culture to induce their predifferentiation into cells with an osteoblast phenotype. The literature indicates that ascorbic acid, dexamethasone, and β-glycerolphosphate among other substances have been used for this purpose.[25]

Although many studies have concentrated on cellular therapies for bone tissue reconstruction, several clinical techniques involving tissue engineering have also been developed to promote periodontal tissue regeneration. To this aim, Tobita et al[26] proved that implanting adipose rat mesenchymal stem cells together with platelet-rich plasma in induced periodontal defects might promote bone and cement tissue regeneration. Best results in that study were obtained at 8 weeks postoperatively, and both newly formed tissues were analyzed through histological and immunohistochemical analyses.

Regenerative medicine has evaluated the use of grafts involving application of undifferentiated mesenchymal stem cells of adipose origin, mostly in orthopedic reconstructions following extensive bone losses due to traumas, infections, tumors, and congenital defects. Arrigoni et al[27] isolated, characterized, and analyzed the differentiation of these cells derived from the adipose tissue of rabbits, rats, and pigs. After exposure to an osteogenic stimulus, these cells exhibited a significant increase in the expression of bone formation markers, such as alkaline phosphate, extracellular calcium deposits, osteocalcin, and osteonectin.

In another study, mesenchymal porcine stem cells were isolated, cultured on a poly-dl-lactic-coglycolic acid matrix, and incubated for 10 days in an osteogenic medium. The samples were then transplanted into surgically induced mandibular bone defects. After 6 weeks, the grafts were histologically, clinically, and radiographically analyzed. The bone defects presented a dense bone tissue repair, showing osteoblasts, osteocytes, blood vessels, and trabecular bone.[28]

As the use of adipose mesenchymal stem cells has been growing substantially in bioengineering lately, additional studies for cell collection are being conducted. Aksu et al[29] compared the osteogenic potential of stem cells collected from human donors' adipose tissue and their relation to gender

and anatomical region. The results indicated that adipose stem cells collected from the abdominal surface of males might present better response in terms of osteoblastic phenotype.

Other donor sites have been suggested and investigated in the literature as an attempt to obtain stem cells in a less invasive way, avoiding greater damage to the donor site. Dental pulp tissue appears to be a promising source of mesenchymal stem cells for tissue engineering.[30] The following dental tissues are considered excellent sources for collecting mesenchymal cells: the dental pulp of impacted third molars, dental pulp of exfoliated deciduous teeth, apical papilla, periodontal ligament, and dental follicles. Cells from these sites may differentiate into several different types of tissues, including bone or nerves.[30–32]

In an experimental study, Honda et al[33] isolated stem cells from the human dental follicle and cultured them in vitro with a subsequent osteogenic induction. They were reinserted into bone defects of rats. After 4 weeks, the bone defects were partially or completely healed, presenting a histological appearance similar to that verified in intramembranous ossifications. Thus, dental follicle cells were identified as promising sources of multipotent precursor cells for organic tissues.

Cellular matrix

An extracellular matrix is an important factor for tissue engineering, as it provides the necessary framework for nutrients, oxygen, and metabolic waste transportation for the cells in the medium.[34] This framework must have properties such as biocompatibility, biodegradability, and nontoxicity to the surrounding cells and tissues and also present a firm consistency so that the final formed tissue may be easily managed during its insertion into the body. When used in tissue engineering, a matrix should also facilitate adherence, migration, proliferation, and differentiation of the cells in question.[35]

Various matrices and cell carriers have been tested as vehicles to recruit and diffuse a sufficient number of cells, cytokines, and growth factors to the site in need of repair and/or reconstruction. Today, a great number of natural and synthetic matrices are commercially available, being biocompatible, biodegradable, and capable of incorporate and diffuse organic molecules and also allowing cellular colonization within their own structure.[34] Physical, chemical, and mechanical structures of the cell carriers are critically important to properly link with the tissue in need of regeneration.[36]

Cell matrices are currently created from a wide variety of materials. Within this group are all allogenous materials, including an allogenous bone matrix, skin and intestinal submucosa; biological polymers such as collagen, hyaluronic acid, and fibrin; ceramic bases or minerals such as tricalcium phosphate, hydroxylapatite, and calcium sulfate; and some metals and alloys, such as titanium.[35] In addition, a variety of synthetic polymers have been applied as matrices, such as the copolymer poly coglycotyde acid.[35]

To fulfill the biocompatibility prerequisites for a cellular matrix, there have been several efforts to develop new materials and compounds. Examination of their structural composition and surface modifications, mostly based in structural and surface nanoscience, have been studied, showing improved preliminary results.[34]

Growth factors in tissue engineering

Platelet-rich plasma (PRP) and its possible benefits in tissue repair have been thoroughly researched in dentistry.[37] Platelet-rich plasma has been employed mainly in alveolar bone grafting for dental implants and periodontal and maxillofacial surgeries.[37] A vast number of scientific studies emphasize the regenerative capacity of PRP based on the presence of various growth factors in its composition. The use of PRP has demonstrated the potential of surgical wound-healing acceleration due to the local release of specific growth factors involved in angiogenesis and collagen production.[16,38]

PRP is biologically composed of plasma, leucocytes, and platelets. The platelets constitute an important element because of their capacity to accumulate and subsequently release growth factors locally at the desired site. These growth factors act as natural mediators that regulate primordial actions over cellular events that take place specifically during tissue repair and/or regeneration. This key role in regulation of cellular events including chemotaxis, cytodifferentiation, DNA synthesis, and extracellular matrix synthesis is well demonstrated in the literature.[39]

By using PRP as a source of growth factors, the following 3 principal proteins located in the alpha-

platelet granules should be addressed: platelet-derived growth factor (PDGF), TGF-β, and insulin-like growth factor (IGF-I).[40]

PDGF presents various positive effects on healing wounds as it stimulates cellular mitogenesis, increases the number of cells responsible for the healing process, stimulates angiogenesis, and regulates the influence of other growth factors. These PDGF properties may allow improvement of the fibroblastic and osteoblastic cellular differentiation and function.[39]

TGF-β1 and TGF-β2 not only initiate bone regeneration but also remain at the healing site, being responsible for the remodeling and maturation of the bone grafts over the mid- and long-term healing processes. The most important function of these 2 growth factors is cellular chemotaxis and the mitogenic capacity of osteoblasts.[39,41]

IGF-I, which is produced by osteoblasts during the collagen type I and II synthesis in bone formation, plays an important role in tissue regeneration. IGF-I increases the amount of reparative osteogenesis and regulates the deposition of the bony matrix by osteoblasts.[39,42]

Bone morphogenic proteins (BMPs) also play a key role in the bone formation process. BMPs are proteins found in high concentrations in bone tissue and are considered responsible for the inductive and regenerative ability of autogenous bone grafts. Currently, a recombinant human bone morphogenetic protein type 2 is commercially available for clinical application in the maxillofacial region (rhBMP-2, Infuse, Medtronic Biologics, Minneapolis, Minn), presented in kits consisting of a diluent and an acellular collagen sponge used as matrix for application according to the need of volumetric bone gain, and presenting promising preliminary clinical results.[43]

The injectable bone technique

Various tissue-engineering techniques have been tested by different investigators over the past years.[44] The injectable bone technique arose as a possible alternative to bone grafting for the regeneration of craniofacial defects. This technique was developed from the ex vivo culturing of mesenchymal stem cells from the patient's bone marrow. Mesenchymal stem cells (MSCs) were cultured and differentiated in vitro into preosteoblastic cells. PRP and a bioceramic base of calcium phosphate and hydroxyapatite were added to the cellular content to form a gel that is applied to the affected bone site.[6,45,46]

During the development of the injectable bone technique, many studies were conducted that yielded promising results. Yamada et al[46] compared bone formation in a study that involved the following 3 grafting techniques in canine mandibles: injectable bone with MSCs and PRP, PRP alone, and particulated autogenous medular bone. Among these groups, the injectable bone group had the highest percentage of newly formed bone.

Another study performed by Ohya et al[47] was conducted in rats and lasted 8 weeks applying the injectable bone technique. The study found that the increase in bone volume in the fourth and eighth weeks was higher in the MSC group with PRP compared with the control group. This was demonstrated by comparing the cortical and particulated medular bone complex, which was also associated with PRP, during the same period. Ito et al[48] obtained better bone hardness results in the MSC-PRP group compared with other tested groups (autogenous bone; bovine bone, Bio-Oss; osseous defect or control group) by using the Vickers hardness test to evaluate the newly formed bone mechanical properties in dogs.

The success of tissue engineering in bone tissue reconstruction has not only been demonstrated in animal studies. Application of the injectable bone technique has yielded a success rate of 100% for dental implants inserted into newly formed bone. The clinical results of a study in 14 patients demonstrated a time reduction in the implant osseointegration period, also suggesting the possibility of implant placement at the same time as the injectable bone in some situations.[49]

Other strategies have been used for bone augmentation in dental implant therapy. The use of distraction osteogenesis, a method for bone lengthening through the fibrocartilage callus growth modulation, which can be combined with tissue engineering, has been applied.[50] Through this combination, Kinoshita et al[51] first isolated and cultivated mesenchymal autogenous stem cells of rats. This cellular compound added with PRP was applied to the distraction site. Through radiographic and histomorphometric analysis, results showed a greater radiodensity and volume of bone formation in tissues that were treated with the osteogenic

material as compared with those treated with PRP alone after previous osteogenic distraction.

Bone regeneration through tissue engineering, an adjuvant in the healing of osteogenic distraction and formation of fibrocartilage callus, has also yielded positive results. Hibi et al[45] reported promising results in a clinical case that combined osteogenic distraction with autogenous osteogenic material. Application of injectable bone to reconstruct the mandible yielded a 3-dimensional bone formation and a shorter fracture healing time. According to the authors, after 3 months, it was possible to insert six 18-mm-long implants to support a fixed dental prosthesis.[51–53]

Implant immediate insertion techniques following dental extractions have been widely used in dentistry. While searching for better esthetic and functional results, bone bioengineering has also been investigated to improve the final bone tissue response. Ito et al[9] presented satisfactory results from simultaneously inserting implants and applying injectable bone for bone regeneration. In their study, the group using MSCs and PRP showed better results compared with other tested grafts. The histological and histomorphometric evaluations revealed significant differences in the bone-to-implant contact ratio, with a greater percentage for the injectable bone technique group after a 1-month healing period.

The rehabilitation of a severely reabsorbed maxilla through osteointegrated implants constitutes a challenging situation in implant dentistry. To repair bone tissue resorption in the posterior maxillary region, the injectable bone technique might be used to elevate the maxillary sinus floor and therefore allow implant placement.[47,49] This technique has also been reported for the reconstruction of maxillary fissures from congenital defects with encouraging results regarding bone regeneration.[54]

Discussion

Various techniques for bone grafting are being widely used in dentistry, applying autogenous, allogeneic, xenograft, and synthetic materials for bone tissue reposition.[1–2,4] However, several limitations involving the use of conventional osseous graft materials are still present, as they lose most of their osteoconductive and/or osteoinductive properties during their processing. Conventional autografts also present considerable disadvantages, including limited available amount of tissue, risk of cross-infection, and tissue damage to the donor site.[55]

Considering the literature mentioned in this review, some challenges and advances have been made in bone reconstruction through tissue engineering. Tissue donor sites with greater abundance of MSCs combined with less-invasive collection procedures are currently being investigated. Zuk et al[19] isolated liposuctioned fat stem cells of mesodermal and mesenchymal origin, which generated different phenotypes when placed in a differentiation-inducing cell culture medium in vitro. Therefore, adipose tissue might constitute an excellent source of multipotent cells, serving as an alternative to bone marrow collection. In addition, in a 10-year literature review, Mizuno[18] stressed the advantages of using mesenchymal adult stem cells autogenously if there were no major ethical or immunoreactive considerations in the collection, culturing, or clinical application processes.

Just as adipose tissue can be a good source of MSCs for tissue engineering, cells from dental tissues such as the pulp and periodontal ligament may also constitute a good alternative for this purpose, exhibiting an expressive osteogenic potential.[30,31,33,56,57]

Growth factors, which constitute another important component of tissue engineering, have also been mentioned in this review. These growth factors act to regulate cellular proliferation and differentiation. Platelet concentrates (PRP), which present these growth factors, were widely used for injectable bone techniques reported in the literature and yielded positive results in experimental groups that combined its use with MSCs.[9,10,55,58]

Considering these growth factors, the clinical use of BMPs may optimize the results of bone reconstruction of the maxillofacial complex, as supported by preliminary results of its application in bone grafts. These proteins belonging to the growth factor beta family (TGF-β),[59] which are obtained by cloning BMPs 2 to 9, are seen today as the most promising resource from the growth factor group to be applied in bone tissue regeneration.

However, a definitive opinion regarding the potential advantages of BMPs for bone regenera-

tion will be possible only following longitudinal evaluations of bone reconstructions performed in duly rehabilitated patients, as this regenerated maxillary bone will truly demonstrate its clinical performance only during long-term functional follow-ups.

Conclusion

The present review has shown the significant progress of cell culture–based tissue-engineering studies, which reported promising preliminary results following the application in bone reconstructions over recent years. Studies reporting the application of the injectable bone technique have demonstrated less tissue-invasive procedures and good bone formation as compared with traditional bone reconstructions; however, there is still a need to optimize clinical techniques and identify alternatives that may apply fewer autogenous components in its manufacturing process. Alternative sources of mesenchymal autogenous stem cells should be identified and tested in all tissue-engineering techniques. An appropriate cell-carrying system that functions as an "ideal matrix," in which these stem cells may proliferate and differentiate into osteogenic cell lines, along with the local delivery of growth factors, might be critical to establish tissue engineering as an effective clinical alternative applicable for the reconstruction of maxillofacial bone defects.

Abbreviations

BMP: bone morphogenic proteins
IGF-I: insulin-like growth factor
MSC: mesenchymal stem cell
PDGF: platelet-derived growth factor
PRP: platelet-rich plasma
TGF-β: transforming growth factor beta

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