

TISSUE ENGINEERING IN PERIODONTAL REGENERATION: A BRIEF REVIEW

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ABSTRACT

Periodontal disease is a major public health issue and the development of effective therapies to treat the disease and regenerate periodontal tissue is an important goal of today's medicine. Regeneration of periodontal tissue is perhaps one of the most complex processes to occur in the body. Langer and colleagues proposed tissue engineering as a possible technique for regenerating the lost periodontal tissues. Tissue engineering is a multidisciplinary field, which involves the application of the principles and methods of engineering and life sciences to help in the development of biological substitutes to restore, maintain or improve the function of damaged tissues and organs. A Google/Medline search was conducted and relevant literature evaluating the potential role of the tissue engineering in periodontal regeneration, which included histological studies and controlled clinical trials, was reviewed. A comprehensive search was designed. The articles were independently screened for eligibility. Articles with authentic controls and proper randomization and pertaining specifically to their role in periodontal regeneration were included. The available literature was analyzed and compiled. The analysis indicate tissue engineering to be a promising, as well as an effective novel approach to reconstruct and engineer the periodontal apparatus. In this article how tissue engineering has advanced and discuss its impact on the clinical management of both periodontal and osseous defects in preparation for implant placement. An understanding of these new tissue engineering techniques is essential for comprehending today's ever-expanding oral plastic surgery procedures.

KEYWORDS: Growth factors; periodontal regeneration; tissue engineering

INTRODUCTION

The ultimate goal of periodontal therapy is to completely restore the periodontal attachment including cementum, periodontal ligament, and alveolar bone lost due to periodontal disease or trauma. In the past few decades, many attempts have been made to unravel the "magic filler" material that could result in new clinical and histological attachment, but have only culminated in healing by repair. Periodontal repair refers to healing that does not allow the original morphological nor functional restoration of the tissue, considered as non-functional scarring. Periodontal regeneration attributes to a complete recovery of the periodontal tissues in both height and function, that is, the formation of alveolar bone, a new connective attachment through collagen fibers functionally oriented on the newly formed cementum.^[1] Tissue engineering was proposed as a possible technique for regenerating lost periodontal tissues by Langer and colleagues in 1993.^[2] Tissue engineering is an interdisciplinary field that applies principles and methods of engineering and the life sciences towards the development of biological substitutes that restore, maintain, and improve the function of damaged tissues and organs.^[3] The goal of tissue engineering is to promote healing, and ideally, true regeneration of a tissue's structure and function, more predictably, more quickly, less invasively, and more qualitatively than allowed by previous passive techniques.^[4,5] The tissue engineering approach to bone and periodontal regeneration combines three key elements to enhance regeneration.^[4]

1. Progenitor cells
2. Scaffold or supporting matrix
3. Signaling molecules

CELL SOURCE FOR PROGENITOR CELLS

Over the last decade, the regenerative capacity of postnatal progenitor cells has increasingly emerged making these cells an attractive candidate for use in tissue-engineering applications. In particular, cell-based periodontal regeneration has been performed using various approaches and principles, and several excellent reviews have been published recently.^[3-5]

Periodontal ligament-derived cells

Because periodontal ligament-derived cells have multipotential characteristics, the cells are regarded as useful sources for the regeneration of periodontal tissues containing bone, cementum and periodontal ligament. Nakahara et al. implanted autologous dog periodontal ligament cells that were seeded onto a collagen sponge scaffold into a periodontal fenestration defect model in dogs, and showed regeneration of alveolar bone and cementum in uniform layers on the root surface.^[5]

Periodontal ligament-derived mesenchymal stromal cells

Seo et al. isolated a population of multipotent stem cells in human periodontal ligament and indicated that periodontal ligament-derived mesenchymal stromal cells exhibited some characteristics similar to those of mesenchymal stromal cells, such as multipotency, clonogenic ability, high proliferation and the expression of the putative stem cell marker STRO-1, as well as the perivascular cell marker CD146.^[6]

Periosteal cells

The cultured periosteum has the capacity to differentiate into an osteoblastic lineage and expresses periodontal tissue related genes. Yamamiya et al. showed cultured periosteum combined with platelet-rich plasma and hydroxyapatite induced clinical improvements in human infrabony defects.^[7]

Gingival epithelium and fibroblast

Gingival epithelial sheets derived from human gingival tissues were developed and applied clinically as a treatment for chronic desquamative gingivitis.^[8] Transplantation of gingival epithelial sheets induced a reduction in inflammation and the gain of a healthy epithelial junction and connective tissue. Mohammadi et al., applied autologous gingival fibroblasts for patients with insufficient attached gingiva and showed the increase in width of keratinized tissue.^[9]

Bone marrow-derived mesenchymal stem cells

Using bone marrow aspirates from over 350 human donors, Pittenger and colleagues (1999) showed lineage specific differentiation of MSCs into fat, cartilage, and bone under appropriate in vitro culture conditions. Not only did the human bone marrow derived MSCs demonstrate ability to extensively proliferate, but these cells also were capable of guided differentiation into multiple cell types, establishing a provocative cell source for potential tissue engineering.^[3] Kawaguchi et al., demonstrated that autotransplantation of bone marrow derived mesenchymal stem cells induced periodontal regeneration in experimental class III furcation defects in dogs.^[10]

SCAFFOLD OR SUPPORTING MATRICES

The major roles for supporting matrices are listed below^[11]

1. It serves as a framework, which maintains the shape of the defect. It provides physical support for the healing area so that there is no collapse of the surrounding tissue into the wound site.
2. It serves as a 3D substratum for cellular adhesion, migration, proliferation and production of extracellular matrix.
3. It serves as a barrier to restrict cellular migration in a selective manner.
4. It potentially serves as a delivery vehicle for growth factors.

BIOMATERIALS USED AS SCAFFOLDS

Ceramics

Natural and synthetic HA (hydroxyapatite) and beta tricalcium phosphate (TCP) are ceramics used in bone tissue engineering. They are biocompatible, osteoconductive and being protein free, they stimulate no immunological reaction. HA (hydroxyapatite) was one of the first biomaterial to be used as a scaffold. It may be derived from bovine bone or coralline or made of a pure synthetic material. TCP is a naturally occurring material comprising of calcium and phosphorous and is used as a ceramic bone substitute.^[12]

Polymers

These include synthetic polyesters, such as polyglycolic acid, polylactic acid and polycaprolactone and natural polymers like collagen fibrin, albumin, hyaluronic acid, cellulose, chitosan, polyhydroxyalkanoates,

polyhydroxyalkanoates, alginate, agarose and polyamino acids.^[12]

Synthetic polyesters

PGA (polyglycolic acid) is a polymer of glycolic acid. It was the first polymeric scaffold used in tissue engineering. It is insoluble in water. It is also used as suture material, and as implants for bone fracture fixation. PLA (polylactic acid) is the polymer of lactic acid. PLA is more hydrophobic than PGA and more resistant to hydrolysis. Copolymers of PGA have been used for many types of biomaterials, including sutures (vicryl). PLGA (polylactic-co-glycolic acid) is a copolymer of PGA and PLA. Due to its biocompatibility, controlled structural and mechanical properties, tailored degradation rates, and its potential as growth factor delivery vehicles, it has been considered as the prime candidate for use in regenerative medicine and dentistry.^[12]

NATURAL POLYMERS

Collagen

Collagen foam

These are fabricated by freeze-drying a solution of collagen and placed in a mold of desired configuration. After physical or chemical cross-linking of sufficient intensity and duration, foam scaffolds become resistant to contraction by tissue cells and exhibit decreased or increased resistance to breakdown by collagenase, depending on the cross-linking regimen.^[13]

Collagen fiber

Fibers with diameters of 300-nm and above have been made on a commercial scale. They can be formed into wools by tangling in a scanning electron micrograph of the wool, into which cells are easily seeded. When cross-linked by methods that do not alter the native 67-nm cross-banding, the fibers are considerably more resistant to collagenase than are foam or gel scaffolds.^[13]

Collagen membrane scaffolds

Collagen membranes can be prepared by allowing collagen in solution to dry on a surface to which it will not bind, like Teflon or polyethylene. To promote formation of fibrils the solution is neutralized and warmed to 37°C, allowing the collagen to polymerize and form the fibrils. Before it begins to gel, the solution is spread on a suitable surface and allowed to dry. Membranes may be cross-linked by a variety of methods to improve their wet strength. For example,

aldehydic cross-linking will prevent cell attachment, and UV cross-linking will reduce resistance to collagenase.^[14]

SIGNALING MOLECULES IN TISSUE ENGINEERING

In order to enhance the *in vivo* efficacy, incorporation of various bioactive molecules into scaffolding materials have been brought into practice. This incorporation facilitates sustained release of bioactive molecules (growth factors) for longer periods of time. Several bioactive molecules have demonstrated strong effects in promoting periodontal wound repair in preclinical and clinical studies.^[14]

PLATELET DERIVED GROWTH FACTOR

Kohler and Lipton (1974) and Ross *et al.* (1974) discovered that the material released from platelets is the principal source of mitogenic activity present in serum, and is responsible for the growth of many cells in culture that are serum dependent. This activity was later localized to the alpha granules within platelets by Witte *et al.* 1978, Kaplan *et al.* 1979 and called platelet derived growth factor (Ross and Vogel, 1978). PDGF is a dimeric molecule comprising of two peptide chains termed as A and B chains. It is considered to be a potent mediator of periodontal tissue regeneration. It is chemotactic and mitogenic for periodontal ligament cells. It stimulates gingival fibroblast hyaluronate synthesis, a pre requisite for the formation of large aggregate of proteoglycans that provide the lattice for extracellular matrix. Furthermore, alkaline phosphatase activity and osteocalcin are down regulated by PDGF, thereby enhancing bone and cementum formation. Samuel E. Lynch (1991)^[15] demonstrated that short term application of the combination of PDGF-B and IGF-I can significantly enhance the formation of periodontal attachment apparatus during early phases of wound healing following surgery. Moon-II Cho (1995)^[16] conducted an animal study on beagle dogs and introduced the "PDGF modulated guided tissue regeneration therapy". He concluded that PDGF-BB modulated therapy promotes periodontal regeneration more rapidly and effectively as compared to GTR alone.

FIBROBLAST GROWTH FACTOR

Fibroblast growth factor is the member of heparin binding growth factor family. There are 7 forms of fibroblast growth factor. It can be isolated from

normal tissues in two forms: Acidic FGF (aFGF) and basic FGF (bFGF). Besides its name its activity exists beyond that of fibroblast and includes a wide variety of cell types such as smooth muscles, endothelial cells, chondrocytes and osteoblasts. Being a mitogen for fibroblasts, osteoblasts, chondrocytes, smooth muscle cells, skeletal myoblast, it has a profound effect on periodontal soft tissue and bone healing. FGF also stimulates angiogenesis, DNA synthesis and cell replication.^[16]

BONE MORPHOGENETIC PROTEINS

These are 15 different types of proteins identified to date as a part of transforming growth factor- β superfamily. Bone morphogenetic protein-2 is a disulfide-linked homodimer. BMP appears to possess a multitude of effects that promote periodontal healing. It helps undifferentiated pluripotent cells to differentiate into cartilage and bone forming cells.^[16] Along with β -FGF, it stimulates angiogenesis. It also stimulates alkaline phosphatase activity, thereby promoting bone formation. Thorarinn J. Sigurdsson (1995)^[17] did a study on beagle dog with artificially created 5 mm deep bone defects and concluded that rhBMP-2 treated sites showed higher alveolar bone level when compared with the control sites.

INSULIN LIKE GROWTH FACTOR

This class of growth factors is also referred to as somatomedins. IGF-I is known as somatomedin C and IGF-II has been called multiplication-stimulating activity. Insulin like growth factor-I is found in substantial levels in platelets and is released during clotting along with the other growth factors. It is a potent chemotactic agent for vascular endothelial cells resulting in increased neovascularization. IGF-1 has strong effect on periodontal ligament fibroblasts mitogenesis and protein synthesis *in vitro*. It promotes osteogenesis and cementogenesis.^[18] IGF-II is the most abundant growth factor in the bone and it also promotes parameters of bone formation but is not as potent as IGF1.

TRANSFORMING GROWTH FACTOR- β

TGF- β was originally identified because it can induce non-transformed cells to grow in soft agar. It is found in highest concentration in bone and platelets. TGF- β is encoded by three different genes TGF- β_1 , TGF- β_2 , and TGF- β_3 . TGF- β is a strong promoter of extracellular matrix

production. It selectively stimulates periodontal ligament fibroblast proliferative activity. It stimulates type I collagen, fibronectin and osteocalcin biosynthesis, as well as bone matrix deposition and chemotaxis of osteoblast. On the other hand, TGF- β decreases synthesis of metalloproteinases and plasminogen activator, and also increases the synthesis of tissue inhibitor of metalloproteinases and plasminogen activator inhibitor (PAI), thus resulting in the decrease of connective tissue destruction.^[19]

PERIODONTAL LIGAMENT DERIVED GROWTH FACTOR

Nishimura *et al.* (1995) isolated a novel polypeptide factor from human periodontal cells periodontal ligament derived growth factor called PDL-CTX. This peptide is highly specific autocrine chemotactic agent for human periodontal ligament cells, which is 1000 fold more potent than many known growth factors (IGF, PDGF, TGF). In addition, PDL-CTX has no chemotactic effect on gingival fibroblast or epithelial cells thereby promising its utility for biological therapeutic regime needed for cell specific periodontal regeneration.^[20]

RECENT ADVANCEMENTS IN TISSUE ENGINEERING

Gene therapy

The single administration of purified tissue growth factors has not been shown to be clinically effective in supporting the horizontal regeneration of periodontal tissue breakdown because of their short biological half-lives. Once applied, these factors are subject to proteolytic breakdown and receptor-binding problems and are dependent on the stability of the carrier system. Gene therapy may circumvent many of the limitations with protein delivery to soft tissue wounds.^[21,22] Gene therapy refers to the treatment of a disease by means of a genetic manipulation. Genetic information is transferred to the target cells, which enables them to synthesize a protein of interest to treat disease. Gene transfer is accomplished through the use of viral [retroviruses, adenoviruses (Ad) and adeno-associated viruses (AAV)] and non-viral vectors (plasmids and DNA polymer complexes).^[23] Gene vectors can be introduced directly to the target site (*in vivo* technique), or selected cell can be harvested, expanded, genetically transduced, and then reimplanted (*ex vivo* technique).^[23]

GENE THERAPY FOR PERIODONTAL TISSUE ENGINEERING

Platelet derived growth factor gene delivery

Plasmid and Ad/PDGF gene delivery have been evaluated in preclinical and human trials. The latter approach has been able to exhibit more safety favorable for clinical use, however.^[24] An *ex vivo* investigation by Anusaksathein and colleagues showed that the expression of PDGF genes was prolonged for up to 10 days in gingival wounds. Ad encoding PDGF B transduced gingival fibroblasts and enhanced defect fill by induction of human gingival fibroblasts migration and proliferation. On the other hand, continuous exposure of cementoblasts to PDGF A had an inhibitory effect on cementum mineralization, possible via the upregulation of osteopontin and subsequent enhancement of multinucleated giant cells in cementum-engineered scaffolds.^[24]

Bone morphogenetic proteins gene delivery

An experimental study in rodents by Lieberman and Colleagues demonstrated gene therapy for bone regeneration, with results revealing that the transduction of bone marrow stromal cells with rh BMP-2 lead to bone formation within an experimental defect comparable to skeletal bone. Another group was similarly able to regenerate skeletal bone by directly administering Ad5/BMP-2 providing further evidence for the ability *ofin vivo* and *ex vivo* bone engineering. Gene therapy presents certain advantages when compared with other therapies. Because both cell transplantation and laboratory cell culturing are not needed, gene therapy may be safer and more cost-effective than cell-based therapies.^[25]

Implantation of live cells

Effective augmentation techniques to treat more challenging esthetic concerns, such as open interproximal spaces and other severe oral soft-tissue deficiencies, though, are not currently available but cell-based therapies may change this. Enumerated below are some of the examples enlightening the use of live cell based therapy in the field of periodontics.^[26]

Treatment of deep periodontal pockets with autologous fibroblasts or placebo

Bowsma and D Souza (2005)^[27] used autologous fibroblasts expanded *in vitro* for soft tissue contouring. Each subject provided a 3mm oral biopsy from which fibroblasts were isolated and expanded. A series of 3 injections of either

autologous fibroblasts or placebo product was made into each study site. Deep periodontal pockets treated with autologous fibroblasts had a statistically significant reduction in pocket depth compared to those treated with placebo.

Use of tissue engineered human fibroblast derived dermal substitute to increase the amount of keratinized tissue

HF-DDS is a tissue engineered human dermal replacement graft manufactured through a three dimensional cultivation of human diploid fibroblast cells on a polymer scaffold. Human fibroblast cell strains are obtained from newborn foreskins and are cultured by standard methods. The fibroblasts remain metabolically active after implantation and deliver growth factors key to neovascularization, cell migration and differentiation. Unlike keratinocytes which carry surface human leukocyte antigens that may cause allograft rejection phenomenon, implantation of allogenic human fibroblasts does not stimulate an immune response. The tissue engineered HF-DDS graft is safe and capable of generating keratinized tissue without the morbidity and the clinical difficulties associated with donor site surgery.^[28]

Bilayered cell therapy: A tissue engineered skin substitute as an alternative to tissue from palate

Bilayered cell therapy is a living bilayered tissue engineered skin substitute constructed of type 1 bovine collagen and viable allogenic human fibroblasts and keratinocytes isolated from human foreskin. BCT is morphologically, biochemically and metabolically similar to human skin. Its cell proliferation rate is similar to that of human skin. Mitotic activity occurs in the basal keratinocytes of the epidermis and in the fibroblasts within the matrix. The keratinocytes produce growth factors and cytokines that act as signals between cells and help to regulate normal wound healing. Bilayered cell therapy exhibits a synergistic interaction between epidermal and dermal layers. It enhances cell and tissue differentiation through cell: Matrix, cell: Cell and cell: Environment interactions. BCT is safe and capable of generating keratinized tissue without the morbidity and potential difficulties associated with donor site surgery (McGuire and Todd Scheyer).^[29]

Human cultured gingival epithelial sheet for promoting tissue regeneration and the concept behind

Momose *et al.* measured the levels of growth factors released from human cultured gingival epithelial sheets (HCGES) into culture medium. Gingival tissues were obtained from patients of generalized chronic periodontitis during periodontal flap surgery. The levels of vascular endothelial growth factor (VEGF), transforming growth factor- α and β 1 (TGF- α and β 1), and epidermal growth factor (EGF) released into the culture medium were determined using enzyme linked immunosorbent assay at just confluent T1 and adequate stratification T2 culture stages. The medium without cells was collected as control (T0). Significantly higher levels of VEGF and TGF- α at T1 and T2 as compared to T0 was found, thereby suggesting the potential of HCGES for promoting wound healing and tissue regeneration after grafting. Besides reducing post-operative discomfort by avoiding the necessity for a tissue graft from the donor site, HCGES being autograft materials, are expected to adhere permanently after grafting because of little influence from immunological rejection. In addition, the availability of frozen cultured epithelial sheets allows several clinical applications for the same patient over a certain period of time from only one biopsy.^[30]

CONCLUSION

To date, a number of studies have reported that progenitor cells, in conjunction with different physical matrices and growth factors, have the capacity to regenerate periodontal tissues *in vivo*. Notwithstanding these significant advances, there are still numerous biological, technical and clinical hurdles to be overcome:

1. Complete understanding about the molecular and signalling pathways that form the ground for tissue engineering and underlie cell renewal and differentiation is yet to obtain.
2. As cell culture medium often requires xenogenic products (such as fetal bovine serum or mouse feeder layers), cell cultures may not be completely free of pathogens and infectious risks are a concern.
3. Culture conditions are not sufficiently developed to mimic the cell microenvironment *in vivo*. The ideal matrix scaffold should mimic native extracellular

matrix, support cell attachment, allow controlled release of bioactive factors, be conducive to tissue in growth and facilitate laboratory handling.

4. Clinical challenges in cell based periodontal therapy relate to immune rejection after administration of stem cells into the host. A potential solution to this problem lies in the use of autologous stem cells to overcome immune rejection.
5. During the extended period in culture, there is a greater likelihood for genetic or epigenetic changes in stem cells. Recently, it was hypothesized that bone-marrow-derived mesenchymal stem cells could be a source of carcinoma-associated fibroblasts (CAF), which has an important role in the growth of epithelial solid tumors. The challenge relating to genomic stability and the risk of tumorigenesis following stem cell transplantation are major safety considerations.

FUTURE PERSPECTIVE

Though, a number of unknowns still remain to be answered, with the continued development of improved methods for gene delivery to cells, as well as advances in our knowledge of the molecular basis of periodontal homeostasis, it is reasonable to anticipate that a simple chairside protocol could be developed in the future. This might involve either the direct delivery of the DNA of interest to the periodontal tissue, or the isolation of a small amount of gingival tissue from the patient, transduction/transfection of the DNA at chairside, and reimplantation of the gene-enhanced cells into the tooth or periodontal ligament space, which might dramatically improve patients' quality of life by providing accurate therapies with fewer side effects, shorter treatment times, and optimal predictability. Further investigation with recombinant growth factors and live cell therapy needs to be conducted to support these methods for everyday clinical practice.

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