

Current Research Directions in Regenerative Therapy

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Abstract

Chronic periodontitis has been the principal cause behind the irreparable destruction of the periodontium. In the field of surgical periodontal therapy, regeneration of damaged tissues by utilizing our body's naturally produced growth factors along with various other biomaterials either synthetically formulated or animally derived has piqued the attention of the dental practitioner. The human body has a natural capacity and potency of regenerating the lost tissue up to an extent; this characteristic is tapped in the regenerative therapy. Presently this therapy is bounded by the limited research and knowledge on the materials that can be used, instruments that are required for a successful procedure and method devised to gain desired outcomes presently available and the body's natural capability for regeneration. Tissue engineering use of stem cell, gene therapy and research on various aspects of regenerative therapy, has produced predicted results in exciting new treatment method for clinicians shortly. Traditional treatment modalities like mechanical instrumentation, curettage and open flap debridement result in keeping the inflammation in check and long junctional epithelium formation which leads to only periodontal repair and not regeneration. Many practitioners frequently use a combined method of putting both barrier membrane and bone grafts to curb the limitations posed by the down growth of epithelium and sagging of the non-rigid barrier used to maintain the grafts in place. This has led to enhanced interest in the Hence, there has been increased attention in the treatment of chronic diseases of the periodontium further elevating the quality of life of the patients. This review article aims to review diverse obtainable bio-materials, scaffolds, barriers and osseous grafts along with the plethora of procedures applied in periodontal regenerative therapy.

Keywords: *Periodontal regeneration, tissue engineering, cell sheet engineering; 3-D printing technology.*

Introduction

The principal aim of regenerative therapy is to fully restore the lost periodontium and other supporting soft and hard tissues that have been destroyed during the chronic inflammatory diseases. The foundation of the

regenerative therapy is built on the formation of the new hard tissues and soft tissue i.e. the alveolar bone proper, the periodontal fibers, cementum along with its associated collagen fibers. It is often observed that traditional periodontal therapy such as scaling and root planing or open flap debridement commonly results in the gain in clinical parameters. It is evidenced by a reduction in the probing pocket depth and clinical attachment gain in the clinical attachment level, but the process of post-operative healing primarily occurs through the formation of long junctional epithelium which is considered as periodontal repair and not periodontal regeneration. The periodontal surgery is critically required for periodontal health and highly expected to regenerate tooth-supportive structures and restore periodontal functions such as pocket reduction procedures and regeneration

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procedures (guided tissue regeneration;GTR or guided bone regeneration; GBR).^{1,2} Adequate blood supply and wound stability, a source of bone and ligament forming cells, a supporting scaffold or matrix, and growth factors to regulate cell migration, proliferation and synthesis and angiogenesis for revascularization of the site are the basic elements required for periodontal regeneration and repair.

Cells in emerging regenerative therapy

Stem Cells:

Among the various types of stem cells found in the oral cavity are

DPSCs: dental pulp stem cells; SHED; PDLSCs: periodontal ligament stem cells; GMSCs: gingiva-derived MSCs, PSCs: periosteum-derived stem cells have reported for to regenerate periodontium. PDLSC differentiate into cells that inhabit and grow on the biocompatible scaffold, for bone tissue engineering in regenerative periodontics.^{3,4} The verification of the osteogenic potential of differentiating PDLSC which is characterized by an increase of nitrous oxide and calcium represents a promising method for the management of periodontal defects. Human PDL has been reported to

be a viable alternative source to be used in stem cell therapies.^{5,6}

MSC niche:this concept is based on the fact that a specialized microenvironment is always desired for mesenchymal stem cells to preserve their ‘stemness’. Under specific signaling conditions. It can differentiate into the endothelial, osteoblastic, adipocytic, odontoblastic, chondrocyte, and neural lineages. The major site of MSC niche in the body is the bone marrow microenvironment.⁷

Cellular Allograft: Novel allografts are cellular. They comprise the body’s mesenchymal stem cells and cells having osteogenesis capacity. These have been successfully used for the management of demanding periodontal defects.⁸

Scaffolds/matrix in emerging regenerative technology: Cell sheet engineering: For enhanced and desirable treatment outcomes, the stem cells require an appropriate medium to reach the target site. This can be made possible by using the scaffolds which aids the stem cells in differentiating into the desired tissues. It also proves to be effective and maybe a promising source of periodontal regeneration.⁹⁻¹¹

Table 1: Review of Literature

Author	Cell sheets	Target site	Results
King GN, et al. 2001 [97]	Human periodontal ligament cell sheet (MCS)in osteogenic differentiation medium	Athymic rat mandible (King’s method)	A layer of cementum & new attachment of collagen fibers to cementum.
Akizuki T, et al. 2005 [98]	PDLSC cell sheets(MCS) using hyaluronic acid as carrier from temperature responsive culture dish	Mesial dehiscence model beagle dogs	Newly formed periodontal ligaments with a rich capillary supply found between alveolar bone & cementum.
Flores MG, et al. 2008 [91]	PDLSC cell sheets(MLS)	Athymic rats	Cementum like hard tissue on dentin surface & Collagen fibres resembling periodontal & sharpey’s fibers were inserted into tissue.
Iwata, et al. 2009[92]	PDLSC cell sheets(MLS) alongwith TCP	Surgically created three walled defects in dogs	New bone and Cementum was formed with well-oriented collagen fibres
Ding, et al. 2010 [99]	Autologous and allogenic PDLSC cell sheets(MLS) alongwith HA/TCP	Periodontal defects in miniature pigs	Significant periodontal regeneration in12 weeks using both autogenous and allogenic PDLSC
Bai, et al. 2011 [100]	DFSCs (MCS) alongwith HERS	Omenta of adult male rats	DFSCs in presence of HERS formed tissues resembling cementum and periodontal ligament
Xei, et al. 2012 [95]	Co-cultured human PDLSC and BMMSC alongwith ceramic bovine bone powder (scaffold based CST)	Athymic rats	Cementum and periodontal ligament like tissue was formed alongwith neovascularization
Na, et al. [87]	CSP of SCAP	<i>In vitro</i>	Increased expression of alkaline phosphatase, bone sialoprotein and runt related gene-2 (RUNX2) mRNA as compared to cell sheets
Zhao, et al. 2013 [86]	CSF of PDLSCs and PRF granules	Reimplantation of teeth in dogs	Better periodontal regeneration and reduced Ankylosis and inflammation
Dan, et al. 2014[88]	MLS composed of cells of gingival connective tissue, alveolar bone and Periodontal progenitors in a calcium phosphate- coated melt electrospun polycaprolactone scaffold	Athymic rats	Scaffold promoted bone formation in 4 weeks. Significant periodontal tissue formed by alveolar bone cells and periodontal progenitors

Shortcomings associated with the cell sheet engineering is that are multiple applications of multi-tiered cell sheets are needed to restore the damaged supporting tissues and make it resemble the original morphology. It is difficult to handle, transport and extremely fragile. Furthermore, this treatment plan is not at all cost-effective and hence only can be carried out in selective cases.

Growth factor-enhanced matrix 21S: It is a synthetic grafting system for bone and periodontal regeneration. It consists of the solution of unaltered recombinant human platelet-derived growth factor (rhPDGF-BB) in its concentrated form. It also has an osteoconductive scaffold (bone matrix). GEM 21S is supplied in a single-use kit. Each GEM 21S kit consists of the following: One cup containing 0.5cc of beta-tricalcium phosphate particles (0.25–1.0 mm). One syringe containing a solution of 0.5 mlrhPDGF-BB (0.3 mg/ml).¹²

PepGen P-15: It is an osseous graft material which has been tissue-engineered. It is composed of the HA bone matrix which is of bovine origin and inorganic in nature. It also consists of artificially formulated cell-binding peptide. This material has been proved to be useful in filling periodontal osseous defects. It has been shown that the P-15 helps in the binding of cells particularly fibroblasts and osteoblasts.¹³ PepGen P-15 bone graft in conjunction with flap surgery was an effective periodontal regenerative potential in terms of linear bone growth and vertical bone growth for the management of three-wall bone defects.¹⁴

Nanomaterials: Nanomaterials available in the forms of nanopowders, nano gels, nano scaffolds are the materials with components less than 100 nm in at least one dimension. these materials can be organic (eg: chitosan, silk fibroin) or synthetic (eg: PGLA nanoparticles, dendrimers).¹⁵ Silk fibroin derived from the cocoons of the silkworm which are processed into scaffolds integrated with bone morphogenetic protein-2 helps in osteogenic differentiation of human mesenchymal stem cells.^{16,17} PLGA nanofibers are biocompatible but its role in bone regeneration can be hindered for its poor osteoconductive.¹⁸

Hydrogels: Fibrin, hyaluronic acid and Pluronic F127 are the hydrogels, which have effective delivery of growth factors. They are the scaffold system which lend a hand in bone repair. However, they are encountered with various drawbacks such as low physical properties such

as mechanical strength, which further decreases their effectiveness as an osseous replacement. This problem can be triumphed over by designing composites which will comprise of an inorganic substance and a copolymer. One such combination is PLGA-g-PEG which has HA and a polyethylene glycol (PEG) as its inorganic substance and copolymer respectively. Its acidic pH environment is neutralized by hydroxyapatite.¹⁹

Titanium mesh: The nonresorbable barrier membranes used in periodontal regeneration are PTFE (expanded or high density) and titanium mesh. Titanium mesh (Ti-mesh) maintain the bone grafts under the barrier and has excellent mechanical properties. Its elasticity prevents mucosal compression. Its enhanced strength and resistance to deformation aids in maintaining the space and also prevent the contour of the tissues from collapsing. Graft displacement is prohibited by its stability. Furthermore, its plasticity allows bending, contouring, and adaptation to any unique bony defect.²⁰

Amniotic Membrane: Amniotic membrane is the deepest lining of the placenta present in the most inner layer of it. It is usually discarded after parturition. It is lately being used in the regenerative and repair therapy both in the medical and dental fields to restore the lost tissues and speed up its repair. It is a novel tissue-engineered biomaterial that has numerous growth factors, proteins and stem cell reserves that help in regeneration of the lost tissues with the acceleration of wound healing. Many studies have also evidenced that this material can be effectively used in the management of intraosseous defects.²¹

Molecular mediators in emerging regenerative therapy: Teriparatide: Teriparatide, is nothing but the parathyroid hormone but in its commercially attainable formulation. It comprises the amino acids starting from 1 to 34 of the parathyroid hormones (PTH 1–34). They are subcutaneous injections, which can be administered by the patient himself in the muscles of the thigh or abdomen daily results into an increase in the number of active osteoblasts, a decrease in osteoblast apoptosis and probably, recruitment of bone lining cells as newly formed osteoblasts, thereby increasing bone strength and tissue integrity which finally leads to bone formation.²²

rhFGF-2: The controlled release, local delivery of growth factors, such as FGF-2 is another emerging approach to regeneration. Recombinant FGF-2 results in broad mitogenic and angiogenic actions that take place

by binding heparin. It helps in the differentiation of osteoprogenitor cells to induce bone formation and also be actively involved in periodontal tissue renewal. 0.3% rhFGF-2 shows a greater capacity for periodontal regeneration than other concentrations.²³

Platelet-derived growth factor: PDGF represents a family of growth factors consisting of 2 polypeptide chains (A & B) which form the dimer (protein pairs).

- PDGF-AA
- PDGF-AB
- PDGF-BB

PDGF-BB can activate any PDGF receptor; therefore its therapeutic application can activate any configurations of its receptors and when used in a synthetic scaffold matrix, promotes long-term stable clinical and radiographic improvements. The proliferation rate of wound fibroblasts is higher by PDGF-BB than PDGF-AB and -AA. PDGF-BB is a synthetic scaffold matrix.²⁴

Concentrated Growth Factor: “Concentrated Growth Factors (GCF)” was introduced in 2006 by Sacco after the concept of PRF. It is a platelet concentrate that belongs to the second generation (advanced). It has many types of growth factors and fibrins. It is achieved by continuously centrifuging the blood and altering the time and the rotations per minute. It enhances the restoration of soft and hard tissues. It is prepared by accumulating 9 ml of patient’s venous blood in the absence of anti coagulating agent in absolutely sterile tubes also referred to as vacuette blood collection tubes. The centrifugation takes place in the following order: first 30 seconds acceleration, next 2 minutes it is rotated at 2700 rpm, next 4 minutes at 2400 rpm, then the following 4 minutes at 2700 rpm, after which for 3 min at 3000 rpm, followed by deceleration for 36 seconds and lastly stopping the centrifugation process. It stimulates the epithelialization process and also increases neo-vascularization making it an exemplary bioactive protein. It also accelerates the alveolar bone healing. In amalgamation with biomaterials and various promising graft materials it aids in the regeneration of the osseous tissues. Acceleration of osseointegration by increased differentiation of osteoblasts increases implant stability. It reduces periodontal intrabony defects depth and mixed with Bio-Oss and shows better results in the early period of healing.²⁵

Biohybrid Interfaces: Modern dental implants

lack the dissipation force, perception, and specialized receptors present in the periodontal ligament. In growing children or patients who are being orthodontically treated osseointegrated implants are not ideally placed due to the absence of a periodontal ligament attachment.²⁶ Hence, a periodontal ligament-attached implant, or “ligaplant,” could be a promising solution to practitioners. Furthermore, it can also provide a mechanism for allowing implant relocation orthodontically. Dental implants coated with HA surrounded with embryonic dental follicle tissues. It is a functional murine ligaplant model which was recently developed by Oshima et al that resulted in regenerated cementum, PDL, and alveolar bone.²⁷

CAD-Based Compartmentalized Designs for Periodontal Complex Formations: In this process 3-D printing technology and computer-aided design (CAD) and is used to create a hybrid scaffold. Sectionalized articulation is present for the hard and soft tissues of the periodontium, including PDL-mimetic architecture which has perpendicular inclination for regeneration of periodontal complex.²⁸

Gene Therapy: This therapy aims at injecting/delivering various growth factors into the defects created due to inflammatory diseases of the periodontium. Numerous methods of administering genes are accessible which offers great flexibility for tissue engineering. Tumor necrosis factor receptor 1 (TNFR1: Fc) is one such soluble protein that is used in gene delivery. It acts as host modulating agent as it curbs the destruction of alveolar bone by decreasing the activity of TNF (tumor necrosis factor). Direct in vivo gene transfer can also be carried out in this therapy. In large periodontal defects, platelet-derived growth factor-B enhance tissue regeneration as demonstrated elsewhere.²⁹ For promising regeneration of osseous defects peripheral to dental implants direct gene transfer of adenovirus/bone morphogenetic protein-7 in a collagen gel carrier has been effective.³⁰

Conclusion

In the present era regenerative therapy continues to face multiple challenges both in research and clinical success. The emerging trends in regenerative therapy has become the turning point in the present-day theory of tissue engineering. However, cells, scaffolds and growth factors used recently have created new possibilities to envision the foresight and prognosis of present-day

surgical therapies. Future research should be carried out to uplift the development of novel treatment strategies. Proper selection of cases, the skill of the practitioner along with proper maintenance by the patient are of paramount importance for the success of regenerative therapy in periodontics.

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