Pathway towards Periodontal Regeneration: A Review

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Abstract

Periodontal regeneration has become one of the primary objectives of periodontal therapy. The resulting scientific endeavours have elucidated modes of periodontal wound healing, the growth of periodontal cells and their association with the surrounding matrix, and growth-promoting factors. The periodontal regeneration industry is producing better and more expensive devices, but the criteria for evaluating their success have not progressed to the same extent. Although clinical measurements of attachment level and probing depths, along with radiography, are good methods of evaluating tooth survival and prognosis, they do not indicate true biological regeneration. The goals of periodontal therapy include not only the arrest of periodontal disease progression, but also the regeneration of structures lost to disease, where appropriate. Conventional surgical approaches (e.g., flap debridement) continue to offer time-tested and reliable methods to access root surfaces, reduce periodontal pockets, and attain improved periodontal form/architecture. However, these techniques offer only limited potential towards recovering tissues destroyed during earlier disease phases. Recently, surgical procedures aimed at greater and more predictable regeneration of periodontal tissues and functional attachment close to their original level have been developed, analyzed, and employed in clinical practice. This article provides a review of the current understanding of regeneration of the periodontium and of procedures used to restore periodontal tissues around natural teeth.

Keyword: Non-bone Replacement Grafts, Bone Replacement Grafts, Gene Therapy, Stem Cell Therapy.

Introduction

Periodontal disease is an inflammatory condition affecting the periodontal tissues that lead to pathological alterations in the supporting tissues which potentially leads to tooth loss.(1) The regeneration of the tooth supporting structures which have been lost as a consequence of periodontal disease progression has been a somewhat elusive goal in periodontics. Though a lot of research has been carried out in an attempt for periodontal regeneration, complete regeneration of the damaged periodontium has still not been achievable. The various approaches used for periodontal regeneration are either conductive or inductive in nature, cell based therapy, gene based therapy and RNA based therapy.(1) The World Workshop in Periodontics(2) defined three regenerative procedures. These procedures are defect debridement by flap curettage, bone grafting and guided tissue regeneration (GTR).(3) To successfully treat periodontal defects, the clinician must understand root and defect anatomy. Variations in root trunk dimensions, root proximity and inter radicular anatomy all may influence the outcome of therapy. Goldman & Cohen (1958)(4) classified intrabony defects according to the number of bony walls surrounding the defects. Three-walled intrabony defects were considered the best defects in terms of anticipated new attachment.(5,6) Techniques currently enjoying widespread clinical usage include bone replacement grafts (BRG), root conditioning with citric acid (CA) or possibly tetracycline (TTC), coronally positioned flap (CPF) and guided tissue regeneration (GTR). Although root conditioning and CPF are typically used in combination with other techniques, BRG and GTR are frequently used as independent approaches. Both BRG and GTR enjoy certain advantages in fulfilling treatment objectives for specific defects.(7) Several factors may account for differences in the success of regenerative therapy. These may include the extent and morphology of the initial pocket and furcation defect;(8) differences in dental plaque control and gingival inflammation;(9) and the extent of bacterial contamination.(10) To understand, what we have to achieve here through periodontal regeneration, there are some terms given below:

1. **Regeneration** refers to the reproduction or reconstitution of a lost or injured part, in contrast to **repair**, which describes healing of a wound by tissue that does not fully restore the architecture or the function of the part.
2. **Periodontal regeneration** is defined histologically as regeneration of the tooth's supporting tissues, including alveolar bone, periodontal ligament, and cementum over a previously
3. Diseased root surface.
4. **New attachment** is defined as the union of connective tissue or epithelium with a root surface that has been deprived of its original attachment apparatus.
5. **Bone fill** is defined as the clinical restoration of bone tissue in a treated periodontal defect. Bone fill does not address the presence or absence of histologic evidence of new connective tissue attachment or the formation of new periodontal ligament.

6. **Guided tissue regeneration (GTR)** describes procedures attempting to regenerate lost periodontal structures through differential tissue responses.

Biological Considerations Relating to Periodontal Regeneration

In the modern era of regenerative biology, pioneering work was carried out in the late 1960s by Tony Melcher, who demonstrated, through his studies, advances in the biology of periodontal wound healing. In 1976, Melcher presented the concept of "compartmentalization," in which the connective tissues of the periodontium were divided into four compartments: the lamina propria of the gingiva (gingival corium), the periodontal ligament (PDL), the cementum, and the alveolar bone. The concept that stem cells may reside in the periodontal tissue was first proposed by Melcher in 1976. In trying to obtain new connective tissue attachment to a periodontally diseased and exposed root surface, several biologic considerations become apparent. First, the reduced periodontium (as a result of the disease process) may have a limited potential for forming the structural components of a periodontium, namely, cementum, periodontal ligament and alveolar bone. Another factor regarding regeneration potential is that the exposed root surface has undergone substantial alterations and changes compared with the situation in health - perhaps these changes inhibit the formation of a new attachment to the affected root surface. Finally, if pocket epithelium is removed during surgical therapy and the connective tissue apposed against the root surface, epithelium tends to migrate between the connective tissue and the exposed root surface, thereby precluding a new connective tissue attachment. Thus, there are several factors which may have a significant biological influence upon the potential for periodontal regeneration.[14]

The Patient Related Factors That Influence Periodontal Regeneration.[1]

1. Diabetes mellitus: studies that demonstrate the physiologic effect of diabetes on periodontal regeneration are lacking. However, Chang PC et al[15] and Shirakata Y et al[16] have confirmed in their animal studies the detrimental effects of diabetes on periodontal tissues and the poor regenerative capacity.
2. 2.Smoking: clinical trials have confirmed that smokers have less reduction in pocket depth, smaller gains in clinical attachment level, less bone fill and greater membrane exposure (GTR) when compared to non-smokers[17,18,19].
3. Biofilm control: poor plaque control and residual periodontal infection are associated with negative outcomes after regenerative therapy.[20]

The Site Related Factors (Mark A Reynolds, Richard T Kao et al 2015[21] includes:

1. **Vertical depth**: Deep and narrow intrabony defects show most significant and predictable outcome. Defects <4mm. in depth may not have favorable prognosis, infact they are more likely to lose than to gain bone.
2. **Defect angle/width**: Narrow intrabony defects are usually self-contained by two or three bony walls and respond to treatment with bone grafts, GTR membrane or biologic agent. However, wider defects require a combination approach. Defects with a radiographic angle of 25º or less (i.e. narrow) gain more attachment. Defects of 37º or more, usually show less gain in attachment.
3. **Number of bony walls:** narrow, deep, 3 wall intrabony defects require a combination approach for regeneration (bone graft + GTR). One wall defects respond less favorably to regenerative therapy. In a combination 1-wall to 2-3 wall defects, the greatest regenerative potential is associated with the 2 and 3 wall component. No predictable regenerative approaches are currently available for pure 0 wall and 1 wall defects.[1]

Techniques for Regeneration

Several surgical techniques have been developed in an attempt to regenerate periodontal tissues:[22]

a. **Non Bone replacement grafts**
1. Curettage and Chemical agents
2. Root surface biomodification
3. Guided tissue regeneration (GTR)
4. Biological Mediators
5. Enamel Matrix Proteins (EMP)

b. **Bone replacement grafts**
1. Autografts
2. Allograft
3. Xenograft
4. Alloplast

c. **Other Techniques:**
1. Gene therapy
2. Stem cells therapy

A. Non Bone replacement grafts

1. Curettage and Chemical agents

Results of removal of epithelium by means of curettage vary from complete removal to persistence of as much as 50%. Therefore, curettage is not a reliable procedure. Ultrasonic methods, lasers, rotary abrasive stones have also been used, but their effect cannot be controlled because of the clinician's lack of vision and lack of tactile sense when using these methods. Chemical agents such as sodium sulphate, antiformin etc. have also been used to remove pocket epithelium but now they are of only historical interest.

2. Root surface biomodification

Root surface conditioning as an adjunct in the treatment of periodontal disease had been proposed

already in the 19th century.[23] Root surface conditioning with tetracycline or citric acid has been used as a part of regenerative procedures[24]. Root surface conditioning was originally suggested because of the ability of acid to modify the root surface by ''detoxifying'' it.[25] Root surface conditioning also showed that collagen fibrils were exposed within the cementum or dentin matrix.[26] Although animal studies demonstrated new connective tissue attachment following acid demineralization, histologic evaluation in human clinical trials demonstrated limited connective tissue attachment and limited regeneration following citric acid demineralization.[27] Blomlof J et al 1997 showed that using EDTA, which has a less acidic pH, may also expose collagen fibers and thus promote cell attachment without having a damaging effect on the surrounding tissues.[28] Results from clinical trials using any type of root conditioning agent indicate no additional improvement in clinical conditions.[29] A meta-analysis systematic review confirmed that the use of citric acid, tetracycline, Hyaluronic acid, Doxycycline, Minocycline or EDTA to modify the root surface provides no clinically significant benefit of regeneration in patients with chronic periodontitis.[30]

3. Guided tissue regeneration (GTR)

This procedure uses barrier membranes to direct the growth of new bone and soft tissue, as it was assumed that periodontal ligament cells are the only cells to have the potential for regeneration.[1] Nyman et al (1982)[51] first reported the application of guided tissue regeneration (GTR) to periodontal defects in humans. The biological principle of GTR is to exclude dentogingival epithelium and gingival connective tissue proliferation into the wound area adjacent to the root surfaces and, simultaneously, to create a space to give preference to periodontal ligament cells for coronal migration (Nyman 1991).[52] The barrier membrane used prevents the epithelial migration into the wound, and also favors the repopulation of the wounded area by periodontal ligament and bone cells. Resorbable and non resorbable membranes are available, Early studies used a Millipore filter, an ePTFE membrane and Rubber dam material has also been used. The resorbable membrane improves problems with the nonresorbable membrane, such as frequent exposure of the membrane, and second surgery to remove the membrane. (Klokkevold PR et al 2006).[53]

Histological evidence of new connective tissue attachment has been presented in animal studies[43] as well as in human case reports following treatment based on the principle of GTR. In addition, gain of clinical attachment level and probing bone level following GTR treatment has been reported in several short-term clinical studies; e.g., by Gottlow et al. 1986[44], Becker et al. 1988[45], Schallhorn and McClain et al. 1988[46], Pontonero et al. 1988[47], Cartellini et al. 1990[48], and in a long-term clinical study by Gottlow et al 1992.[49] It can thus be concluded that regeneration of the periodontium; i.e., the formation of new cementum with inserting connective tissue fibers as well as new bone formation, can be accomplished if the treatment procedure is based on the biological principle of guided tissue regeneration.[50]

Non-Resorbable Membranes

When ePTFE membranes were used in controlled clinical trials treating mandibular Class II furcation defects, significant clinical improvement has been noted. Treatment of maxillary Class II and mandibular Class III defects with such membranes have also reported clinical improvements, but of a more modest and unpredictable degree.

Results using ePTFE to treat intraosseous defects show substantial bone fill averaging approximately 3.0 to 5.0 mm either with or without augmentation with graft materials.

Bioabsorbable Membranes

Non-resorbable membranes require a second surgical procedure with patient discomfort and membrane exposure, leading to bacterial colonization so, these factors have led to the development and utilization of various absorbable membranes for GTR procedures. Polylactic acid and Collagen membranes have been shown to be as effective as other GTR membranes in inhibiting epithelial migration and in promoting new connective tissue attachment.

4. Biological Mediators

Growth factors are polypeptide molecules released by cells in the inflamed area that regulates events in wound healing. These are proteins responsible for coordinating cellular repair processes. Growth factors therapy aim to stimulate the specific progenitor cells which are responsible for the regeneration of mineralized and non-mineralized tissues that comprises the periodontium. As natural biological mediators, polypeptide growth factors modulate significant cellular event in tissue repair, like:

Cell proliferation

Chemotaxis or directed migration

Differentiation

Matrix synthesis via binding to specific cell surface receptors.

Currently the growth factors which are believed to contribute to periodontal regeneration include:

a. Platelet derived growth factor (PDGF)
b. Insulin like growth factor (IGF-I and IGF-lI)
c. Fibroblast growth factor (FGF)
d. Transforming growth factor (TGF alpha and beta)
e. Bone morphogenic protein (BMP 1-12)
f. Epidermal Growth factor (EGF)

These mitogenic polypeptides attract mesenchymal cells and fibroblasts to migrate into the periodontal

wound and stimulate their proliferation.[55] The continuing process of periodontal tissue repair is followed by granulation tissue as a source for future periodontal connective tissue cells such as osteoblasts, PDL fibroblasts and cementoblasts[22].

5. **Enamel Matrix proteins**

Enamel matrix derivative (EMD) or enamel matrix protein, mainly amelogenin, is secreted by Hertwig's epithelial root sheath during tooth development. It is a semipurified protein which contains a mixture of low molecular weight proteins. It was first introduced and marketed as Emdogain in 1996. Evidence suggests that EMD when applied onto root surfaces, gets absorbed into the hydroxyapatite and collagen fibers, in which they induce cementum formation followed by periodontal regeneration. EMD alone or in combination with graft materials provide clinical outcome and long term clinical stability[54] (Espsito M et al 2009).

With the advent of the use of platelet-rich plasma (PRP) to promote regeneration of connective tissue[35], the importance of a stable blood clot for successful periodontal regeneration has been recognized. In fact, it has been suggested that PRP, in conjunction with bone and periodontal regenerative therapy, may promote faster healing, which has led to the development of expensive chairside platelet-purifying centrifuges.[36] It has been claimed that the PRP generated by these units acts as a source of factors that accelerate and improve healing and regeneration (e.g., transforming growth factor-beta [TGFβ1] and platelet-derived growth factors [PDGF]).[37] However, the notion that PRP increases levels of TGFβ1 and PDGF must be examined carefully.

Given that these cytokines modulate and stimulate osteodifferentiation and osteogenesis by serving as chemoattractants and differentiation-stimulating factors for mesenchymal cells[38], their clinical use should theoretically be beneficial, but this may not be the case in practice. Notably, these cytokines have been shown to have biphasic effects on mesenchymal cells both in vivo and in vitro. In this regard, TGFβ1 and PDGF can also inhibit osteogenic cell differentiation[39], an effect that appears to depend on dose and mode of administration. Indeed, given the vagaries of and variations in the clinical methods for preparing PRP, as well as the limited understanding of the underlying mechanisms of its action, there may be little need for fresh platelet products or the expensive chairside machines used to prepare them[40].

b. **Bone replacement grafts**

Bone replacement grafts, such as autografts, allografts, xenografts, and alloplasts, remain among the most widely used therapeutic strategies for the correction of periodontal osseous defects.[31]

1. **Autogenous Grafts**

The need for progenitors, blood supply and morphogens has encouraged the use of autogenous osteogenic tissue for grafting. For example, osseous coagulum and bone blend[32] has been and still is used to achieve bone filling in periodontal and osseous defects. The rationale for the use of this mixture as well as blood and osteogenic cells is to supply progenitors and morphogens to the wound site and to promote stable clot formation. Histological analyses of tissues produced following these procedures have confirmed cementogenesis, osteogenesis and re-formation of functionally oriented ligament fibres, even on root surfaces covered with infected accretions.[33] Notably, even with autogenous grafts, the formation of functional periodontal fibres and new cementum is limited and generally occurs at the very base of the defect, where the conditions are apparently more conducive to regeneration (e.g., in the proximity of a vital periodontal ligament). Moreover, certain types of bone, such as fresh iliac marrow grafts, contain osteoclastic precursors that can promote root resorption and ankyloses.[34]

2. Allogenic Bone Grafts

There are several types of bone allografts available from commercial tissue banks which include iliac cancellous bone and marrow, freeze-dried bone allografts, and decalcified freeze-dried bone allografts. The role of allogenic bone grafts in periodontal regeneration has been recently reviewed by Reynolds et al 2003. Controlled clinical trials indicate bone fill ranging from 1.3 to 2.6 mm when freeze-dried bone allografts (FDBA) were used to treat periodontal defects.

3. Xenografts

Other types of bone substitutes used for grafting around periodontal defects include xenogenic materials like Calf bone (Boplant), Anorganic bovine- derived bone Bio-Oss (OsteoHealth). A xenograft (heterograft) is a graft taken from a donor of another species.[41] These grafting materials are also referred to as anorganic bone, since proprietary processes are suggested to remove all cells and proteinaceous material, leaving behind an inert absorbable bone scaffolding upon which revascularization, osteoblast migration, and woven bone formation supposedly occur.[42]

4. Alloplasts

An alloplast is a synthetic graft or inert foreign body implanted into tissue. Six basic types of alloplastic materials are commercially available: nonporous hydroxyapatite (HA), hydroxyapatite cement, porous hydroxyapatite (replamineform), beta tricalcium phosphate, PMMA and HEMA polymer (a cal calcium layered polymer of polymethylmethacrylate

and hydroxyethylmethacrylate), and bioactive glass. It has been reported that porous and non-porous HA materials and PMMA and HEMA polymer are nonresorbable while tricalcium phosphate and bioactive glass are bioabsorbable.

c. Other Techniques:

1. Gene Therapy

It involves the transfer of genetic information to target cells which enables them to synthesize a protein of interest to treat disease. According to Encyclopedia Britannica 1998 there are 3 types of delivery systems, a viral vector, a chemical method or a physical method. The most common and accurate method is viral vector. They deliver the desired gene to a target cell. Viral vectors are Retro virus, Adenovirus and non viral vectors are plasmid or DNA polymer complexes.[22]

2. Use of Stem Cells

Tissue engineering using mesenchymal stem cells (MSCs) includes high quality regeneration of damaged tissues without forming fibrous tissue, minimum donor site morbidity compared to autografts, low risk of autoimmune rejection and disease transmission. MSCs were first identified by Friedenstein et al. in 1966 from bone marrow.[56] In vitro biological properties of highly purified mesenchymal progenitor cells (MPCs) harvested from the PDL of deciduous and permanent teeth are comparatively assessed. MPCs have also been isolated from the dental follicle of human 3rd molars. It is believed that periodontal regeneration can be successfully attained through the migration of periodontal ligament stem cells and these cells can subsequently get differentiated into osteoblasts, cementoblasts and fibroblasts.

Conclusion

Regeneration of lost supporting tissues remains a primary goal in periodontal therapy. Recent advances are affording new vistas in retaining teeth previously considered hopeless. Regenerative techniques are also the only approaches depicting improvement and stability in furcation defects that are recalcitrant to other modes of periodontal treatment, including nonsurgical therapy, tissue attachment therapy and respective therapy. Although the literature on combined regenerative therapy is scarce, it can be anticipated that this treatment approach will increase in predictability and application as additional information becomes available to further enhance the healing dynamics. It is important to understand the various limitations in the assessment of periodontal regeneration, such as confirming the formation of bone rather than ectopically mineralized fibrous tissues, as well as the re-formation of the attachment apparatus after therapy. Moreover, as stated above, even with the "best" regenerative treatments available, it is probably appropriate to overcome the clinical impulse to fill or regenerate every defect, so that simpler approaches to controlling disease, which have greater evidence for long-term success can be used.

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