# Group C
# Initiator Paper
# Periodontal regeneration - fact or fiction?

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## Introduction

Periodontal disease is an all-encompassing term relating to inflammatory disorders of the periodontium, which range from the relatively benign form known as gingivitis to the more aggressive forms of early onset periodontitis and rapidly progressive periodontitis. All forms of inflammatory periodontal diseases are associated with bacterial deposits on the root surfaces (Offenbacher *et al.,* 1996; Page *et al.,* 1997). One of the most significant outcomes of periodontal inflammation is connective tissue damage. Because the gingival tissues have a remarkable capacity to regenerate to their original form and function, the tissue damage caused by gingivitis is reversible, provided the causative agent(s) are removed (Melcher, 1976). However, with long-term plaque deposition the disease may become more established and destructive. Depending upon host, genetic, environmental and other factors, there may be subsequent loss of connective tissue attachment to the root surface, bone resorption, and formation of a periodontal pocket. In contrast to gingivitis, with the establishment of periodontitis, many of the architectural changes to the hard and soft connective tissues are irreversible - even if the causative inflammation is controlled.

One of the goals of periodontal therapy is to restore periodontal tissues affected by disease to their original architectural form and function. This requires regeneration of the gingival connective tissues destroyed by inflammation, formation of cementum, restoration of lost bone, and re-establishment of connective tissue fiber attachment into previously diseased root surfaces. However, predictable and complete regeneration of the diseased periodontium has been difficult to achieve. Nonetheless, since the pioneering guided tissue regeneration experiments of just over 30 years ago (Nyman *et al.,* 1982), studies have repeatedly demonstrated that periodontal regeneration is biologically possible and clinically feasible.

### *What is regeneration?*

To understand the outcomes of periodontal therapy precisely, the following terms have been defined (The American Academy of Periodontology, 1992): *Periodontal repair* is the restoration of new tissue that does not replicate the structure and function of lost tissue and is analogous to scar tissue formation. *Periodontal regeneration* is defined histologically as regeneration of the tooth's supporting tissues, including alveolar bone, periodontal ligament and cementum over a diseased root surface.

It should be noted that the full extent and success of periodontal regeneration must be assessed not only using clinical parameters (periodontal probing, radiographs), but also re-entry evaluations and, ideally, histological confirmation (Bosshardt and Sculean, 2009). Clearly such assessments are not always possible, and so there has been great reliance upon animal models and some rare human histology studies.

There are at least four criteria that must be met in order for periodontal regeneration to have occurred. These include all the features of the normal dentogingival complex that would equate to restoration of these tissues to their original form, function and consistency (Bartold *et al.,* 2000):

1. A functional epithelial seal must be re-established at the most coronal portion of the tissues and be no more than 2 mm in length.
2. New connective tissue fibers (Sharpey's fibers) must be inserted into the previously exposed root surface to reproduce both the periodontal ligament and the dentogingival fiber complex.
3. New acellular, extrinsic fiber cementum must be reformed on the previously exposed root surface.
4. Alveolar bone height must be restored to within 2 mm of the cemento-enamel junction.

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### *Processes involved in periodontal regeneration*

The processes involved in periodontal healing are largely the same as those for other organs and tissues. However, there are some significant differences. Inflammation is a major requirement: the formation of a blood clot at the site to be healed is needed to provide a provisional matrix, which subsequently becomes organized into granulation tissue. Formation of granulation tissue and fibroblast proliferation are features of chronic periodontitis associated with healing and repair. The granulation tissue is subsequently remodelled into scar tissue or regenerated tissue. Periodontal regeneration is unique because it involves both soft (gingival and periodontal ligament) and mineralized (bone and cementum) connective tissues. The healing of all periodontal components needs to be coordinated and integrated in order for regeneration to occur. Many molecules and cell types presumably participate in this process. The cellular events required are migration of cells by chemotaxis, their adhesion, proliferation, differentiation and production of matrix components. One of the crucial cellular events is recruitment of cells through which the selected cell type determines whether healing occurs by repair or regeneration. The mechanisms involved in cell selection are still largely unknown, although it is likely to involve selective chemotaxis, adhesion, and specific cell-molecular interactions.

## Historical perspective of regenerative procedures

### *Root surface conditioning*

Over the years many techniques have been used in an attempt to increase the amount of connective tissue reattachment to tooth roots following periodontal treatment. Most of these have focussed on trying to improve the biological compatibility of the root surface to new connective tissue attachment. These concepts were founded in the belief that the root surface must serve as a suitable site for cell attachment and fiber development during regeneration. Furthermore, it was considered that diseased root surfaces were contaminated by bacterial products, and were characterized by loss of collagen, alterations in mineral density, and composition of the surface. Accordingly it was proposed that these surfaces did not support the attachment or growth of fibroblasts but promoted epithelial migration along the surface. For these reasons, attempts were made to modify the diseased root surfaces to make them conducive for the attachment of connective tissue cells. A list of root surface conditioning agents investigated over the years is detailed in *Table 1*. Despite the apparent sense in trying to improve the biological compatibility of the root surfaces using such conditioning agents, clinical results were disappointing (Fuentes *et al.,* 1993; Mariotti, 2003; Moore *et al.,* 1987). Irrespective of the type of demineralizing agent used, it cannot be claimed that demineralization of the root surface *per se* is a regenerative procedure. It may, however, have a positive effect on wound healing and be used as a component of, or a step within, various regenerative procedures.

**Table 1.** Agents used for root surface conditioning

| | |
|---|---|
| Acid etching | Citric acid<br>Tetracycline |
| Detergents | Cetylpyridinium chloride<br>Sodium N-lauroyl sarcosine |
| Chelating agents | Ethylenediaminetetraacetic acid<br>Egtazic acid |
| Enzymes | |
| Attachment proteins | Fibronectin |
| Growth factors | |

### *Graft materials used for periodontal regeneration*

Regeneration of bone defects associated with periodontal disease and restoration of architectural form of the alveolar arch due to tooth loss remains a significant problem in dentistry. Ingrowth of soft connective tissue into such defects often occurs and this prevents the formation of new bone tissue, causing aberrations and functional disturbances. In an effort to stimulate osteogenesis, various grafting procedures and materials have been developed. However, the search for an ideal graft material continues to be a challenge. A list of some graft materials investigated is shown in *Table 2*.

There have been several philosophies developed regarding the importance of repair of bony defects caused by chronic periodontal diseases. The rationale behind the use of bone grafts in angular bony defects is that the presence of bone tissue close to a scaled and root planed surface would stimulate the formation of a connective tissue attachment. However, such concepts have been disputed because, on biological grounds, the use of bone transplants for the management of periodontal defects is highly questionable (Karring *et al.,* 1984).

**Table 2.** Grafts and biomaterials used for periodontal regeneration

| | |
|---|---|
| Grafts | Autogenous bone<br>Allogeneic bone<br>Xenogenic bone |
| Biomaterials | Beta tricalcium phosphate<br>Hydroxyapatitie<br>Calcium sulfate<br>Calcium phosphate<br>Bioactive glass |

Boyne (1973) stated that an ideal graft material should: (1) exist in an unlimited supply, without the need for violation of a distant donor site; (2) provide immediate osteogenesis for rapid consolidation; (3) elicit no adverse host responses such as immune reaction; (4) facilitate re-vascularization, which assists early healing and resistance to infection; (5) stimulate osteoinduction of recipient site cells; (6) be adaptable to a variety of physical requirements; (7) cause no impediment to growth or orthodontic tooth movement; (8) provide support and stability where discontinuity or mobility exists; (9) provide a framework for osteoconduction; and (10) be completely replaced by host bone of the same or superior quality and quantity as quickly as possible. In light of our current understanding of the contribution of specific components of the periodontium, for periodontal defects, an ideal graft material should also induce or enhance cementogenesis and the formation of a new attachment apparatus (new bone, cementum, and periodontal ligament). To date there is no such material that fulfills these requirements.

Many osseous grafting materials have been used to try to promote periodontal regeneration and have included autografts, allografts, xenografts and alloplastic materials.

The use of various grafting materials may produce radiographic evidence of bone fill and clinical evidence of improvement in probing depths and clinical attachment levels. However, several studies have shown that, even though there is some bone growth around the graft particles, there is substantial fibrous encapsulation of the graft (Becker *et al.,* 1996; Xiao *et al.,* 1996). In addition, there is an interposed layer of epithelial cells present on the root of the tooth, and consequently, no connective tissue reattachment (Yukna, 1976; Listgarten and Rosenberg, 1979; Caton *et al.,* 1980). Consequently, the relevance of these materials to the regeneration the periodontium can be questioned.

### *Evidence to date regarding grafting materials for periodontal regeneration*

Several systematic reviews have concluded that the use of grafting materials for the management of class II furcations may result in improved probing depth reduction and gains in clinical attachment levels compared with open flap debridement (Trombelli *et al.,* 2002; Reynolds *et al.,* 2003). However in terms of regeneration it is clear that bone fillers generally result in bone deposition adjacent to pre-existing bone. Further away from the pre-existing bone edge, simple bone fillers without the presence of any osteoinductive agents are usually engulfed by fibrous connective tissue. Interestingly, this response does vary among various bone filling agents. For example, BioOss® (Geistlich, Wolhusen, Switzerland) tends to show less fibrous encapsulation compared to bioactive glass and biphasic calcium phosphate (Sculean *et al.,* 2004; Windisch *et al.,* 2008). Furthermore, it must be noted that most of the responses noted to date are limited to bone regeneration with little regard for new cementum and new periodontal ligament formation. Indeed, histological evidence for new connective tissue attachment to root surfaces following the implantation of these graft materials is very limited. Similar findings have been noted for autogenous bone grafts (Wang *et al.,* 2005). The outcomes following the use of allogeneic bone grafts in periodontal defects have also been extensively reviewed (Wang *et al.,* 2005). There appears to be little good evidence that the use of allogeneic materials results in any significant periodontal regeneration (Dragoo and Kaldahl, 1983; Bowers *et al.,* 1989a, Bowers *et al.,* 1989b; Bowers *et al.,* 1989c).

## Guided tissue regeneration

### *Principles*

By the early 1980's a number of important observations had been made in relation to periodontal regeneration. These have been summarized by Card *et al.* (1987) and are presented here in a slightly modified form:

1. Regeneration is biologically possible but can only be verified by histological analysis.
2. Epithelial migration and formation of a long junctional epithelium is a fundamental healing process occurring after either surgical or non-surgical periodontal therapy.
3. Formation of a long junctional epithelium prevents root resorption by gingival connective tissue but also impedes new connective tissue attachment onto the root surface.
4. Periodontal ligament cells colonize root surfaces quicker than bone-derived cells, thus preventing ankylosis.
5. New attachment can be obtained onto a root surface that has been exposed to the oral environment.
6. Gingival connective tissue cells and bone cells do not appear to have the ability to form new connective tissue attachment onto a root surface.
7. Cells derived from the periodontal ligament appear to have the potential to form new connective tissue attachment onto a root surface.
8. In order for regeneration to occur, selective repopulation of the wound site must occur with cells possessing the potential to form new cementum, periodontal ligament, and alveolar bone.

From the above observations, a clinical procedure based on the principles of guided tissue regeneration was developed (Nyman *et al.,* 1982b). This method relies on draping a barrier membrane from the root surface over the periodontal defect and onto adjacent alveolar bone prior to replacement of a full thickness mucoperiosteal

flap. In doing so, a space is provided into which cells from the periodontal ligament may migrate, and an effective barrier prevents either gingival connective tissue or epithelium from occupying this space during the healing (regenerative) phase. Apart from exclusion of gingival epithelium and connective tissue from the healing site, wound stability, adhesion of the blood clot to the tooth surface, and the provision of adequate space by the barrier membrane are also considered to be contributory to the successful outcome of guided tissue regeneration therapy (Scantlebury, 1993).

### *Types of membranes*

A wide range of materials, including methylcellulose acetate, expanded polytetrafluoroethylene (ePTFE; GORE-TEX®, Gore, Flagstaff, AZ, USA), collagen, polyglycoside synthetic polymers, and calcium sulfate have been tested for effectiveness and used as a physical barrier in guided tissue regeneration. These membranes are derived from a variety of sources, natural and synthetic, and are either bioabsorbable or nonresorbable (Villar and Cochran, 2010; Bottino *et al.,* 2012).

### *Non-resorbable membranes*

Prior to commercialization of guided tissue regeneration membranes, the original experiments in this field used Millipore filters (type GS: Millipore A, 67 Mosheim, France: pore size 0.22 μm; Nyman *et al.,* 1982b). However, these membranes were fragile and tended to tear, which limited their clinical use. Methylcellulose acetate barriers were later replaced by non-resorbable ePTFE membranes specifically designed for periodontal regeneration. A specific design feature of the ePTFE membranes was an open microstructure collar and a cell occlusive apron. The collar was designed to impede epithelial downgrowth and the occlusive apron was designed to inhibit gingival epithelium and connective tissue gaining access to the repairing periodontal defect. This material not only possesses adequate stiffness to allow creation and maintenance of a secluded space into which the new attachment will form, but also is supple enough to allow adequate adaptation over the defect (Villar and Cochran, 2010). Non-resorbable membranes are particularly prone to exposure to the oral environment. As a consequence, bacterial contamination and infection may result in delayed wound healing and poor regenerative outcomes. These membranes need to be removed after 6 to 8 weeks in a second surgical procedure.

### *Resorbable membranes*

Various bioabsorbable materials, including polyglycoside synthetic polymers (i.e., polymers of polylactic acid, polyglycolic acid, polylactate/poly-galactate), collagen, and calcium sulfate have been developed as membrane barriers. The clinical efficacy of bioabsorbable membranes depends on their ability to retain their structural physical integrity during the first 6 to 8 weeks of healing and to be gradually absorbed thereafter. Based on this concept, chemicals and structural modifications (i.e., polymerization, cross-linking) were incorporated into bioabsorbable membranes to extend their absorption time and increase the clinical effectiveness of these materials.

Biodegradable collagen membranes possess a lower risk of exposure and do not need a second surgical procedure for their removal. As collagen membranes possess fewer favorable mechanical properties than non-resorbable membranes, bone filler is needed to prevent their collapse into the defect area.

Chitosan, a deacetylated derivative of chitin, is another biomaterial used for guided tissue regeneration that is biodegradable. Its property of bacteriostasis may reduce the bacterial contamination and benefit periodontal tissue regeneration (Xu *et al.,* 2012).

In the future there will be further developments in the rational design of biodegradable products for guided tissue regeneration. It is likely these developments will include enhanced mechanical properties with controlled degradation dynamics together with delivery systems for bioactive and antibacterial agents (Bottino *et al.,* 2012).

### *Does guided tissue regeneration work?*

The guided tissue regeneration technique is sensitive and technically demanding. From the studies published to date it is apparent that gains in both probing and attachment levels can be expected following guided tissue regeneration procedures, although there is significant variability depending on numerous clinical parameters that affect periodontal regeneration outcomes (Demalon *et al.,* 1993; Tonetti *et al.,* 1993; Mellonig *et al.,* 1994; Machtei *et al.,* 1994). Some of the major limiting factors include: defect size and location, type of furcation defect, degree of membrane exposure during healing period, and degree of microbial contamination. A more recent systematic review confirmed that the clinical outcomes of guided tissue regeneration on parameters such as attachment gain, reduced pocket depth and hard tissue gain at re-entry surgery are all greater than open flap debridement (Needleman *et al.,* 2005). However, it was noted that there was marked variability among studies and the clinical relevance of these improvements was unclear.

Long-term studies and evaluations of guided tissue regeneration have indicated that the clinical improvements obtained by this procedure are of small magnitude and exhibit large variability (Bratthall *et al.,* 1998; Pontoriero and Lindhe, 1995; Wallace *et al.,* 1994; Machtei *et al.,* 1996). Two meta-analyses have concluded that guided tissue regeneration yields greater clinical attachment gain than open flap debridement alone for intrabony

and furcation defects (Jepsen *et al.,* 2002; Murphy and Gunsolley, 2003). In addition, quantitative analyses of clinical outcomes following guided tissue regeneration treatment suggests that this therapy is only a successful and predictable alternative in well-selected cases such as narrow intrabony defects and class II mandibular furcations (Villar and Cochran, 2010). Notwithstanding the generally modest gains in clinical attachment, 10-year follow-up studies demonstrate stable gains in clinical outcomes and thus support the use of guided tissue regeneration in treatment of infrabony periodontal defects and class II furcation defects (Eikholz *et al.,* 2006; Pretzl *et al.,* 2009; Nickles *et al.,* 2009; Sculean *et al.,* 2008a). However, for more advanced defects such as class III furcations and 1-wall infrabony defects guided tissue regeneration does not result in very predictable outcomes (Gottlow *et al.,* 1992; Becker and Becker 1993).

While the histological outcomes of new attachment, new cementum and new bone formation are well documented in animals, the outcome is less well documented for humans. At the time of membrane removal (for non-resorbable membranes), the regenerating tissues forming underneath the membrane are of a soft, gelatinous consistency (Becker *et al.,* 1988). With time, this tissue may mature into bone - although this appears to be a rather variable response. All of the human histological studies to date have been either case reports or case series on very low numbers of subjects under non-standardized experimental conditions (Nyman *et al.,* 1982; Gottlow *et al.,* 1986; Stahl *et al.,* 1990; Sculean *et al.,* 1999; Stoller *et al.,* 2001; Windisch *et al.,* 2002). These studies have indicated that the predominant healing process following guided tissue regeneration procedures is via new connective tissue attachment to the root surface with minor contributions of new cementum and bone formation. Therefore, by definition, regeneration has not occurred.

## Combinations of guided tissue regeneration and bone grafts

One challenge of regenerative therapies has been to achieve alveolar bone replacement in furcation, dehiscence, and horizontal defects coronal to the existing bony crest level. Guided tissue regenerative techniques alone have failed to achieve this. More recently, a combination of grafting treatments and barrier membranes has been attempted to augment the technique of guided tissue regeneration. Often these were combined with root demineralization techniques. The combinations include resorbable or non-resorbable barrier membranes with bone graft or synthetic grafts placed under them, and coronally positioned flaps. Several studies have reported some improvement in the healing of furcation defects when a combination of guided tissue regeneration membranes and demineralized freeze-dried bone allografts or dura mater membrane were used (Anderegg *et al.,* 1991; Schallhorn and McCain 1988; Zaner *et al.,* 1989). However, these assessments were based solely on clinical criteria and no histological data were available. More recently, the effects of guided tissue regeneration, with and without demineralized freeze-dried bone allografts, in the treatment of furcation defects in dogs with naturally occurring periodontal disease, has been evaluated (Caffesse *et al.,* 1993). In this histological study, adjunctive bone grafting did not appear to enhance regeneration. In a human study, comparing demineralized freeze-dried bone allografts with and without ePTFE membranes in periodontal defects and using allografts as controls, it was concluded that utilization of ePTFE membranes, in addition to demineralized freeze-dried bone allografts, did not lead to additional radiographic gains in the defect area (Guillemin *et al.,* 1993). However, a relatively recent systematic review came to the conclusion that most preclinical studies have histologically demonstrated periodontal regeneration when grafting materials are combined with barrier membranes (Sculean *et al.,* 2008b). Thus, the overall conclusion of these studies is that the results for combined guided tissue regeneration and grafting materials are variable and benefits, if any, are only marginal.

## Biological agents for periodontal regeneration

With the limitations of the above agents and procedures in mind, more recent efforts in periodontal regeneration have been focused on the use of biological agents to assist in stimulating self-repair/regeneration mechanisms within the periodontium. This approach has been referred to as "endogenous regenerative therapy" (Chen *et al.,* 2010), and is an important and exciting emerging area in periodontal regeneration. This field focuses on the use biological agents such as growth factors, matrix extracts, plasma concentrates and biologically active peptides to stimulate the host's inherent capacity for periodontal regeneration.

### *Enamel matrix proteins*

An important advancement in periodontal regeneration was the discovery of enamel matrix proteins, produced by Hertwig's epithelial sheath (Lindskog, 1982; Slavkin *et al.,* 1989). These proteins were shown to play an important role in cementogenesis, as well as in the development of the periodontal attachment apparatus (Ten Cate, 1996; Hammarström, 1997). This observation led to the development and utilization of the biologically active agent "enamel matrix derivative" (EMD, Emdogain; Straumann AG, Basel, Switzerland) as a local adjunct to periodontal surgery for stimulating regeneration of periodontal tissues (Hammarström *et al.,* 1997; Wilson, 1999; Rathe *et al.,* 2009; Sculean *et al.,* 2007a; Sculean *et al.,* 2007b; Venezia *et al.,* 2004).

Numerous clinical and histological studies have demonstrated that treatment of periodontal defects with EMD results in periodontal regeneration (Esposito *et al.,* 2005; Venezia *et al.,* 2004; Kalpidis and Ruben, 2002; Esposito *et al.,* 2009; Rathe *et al.,* 2009; Sculean *et al.,* 2007c). In addition, several systematic reviews evaluating the results of randomized clinical trials have confirmed the positive clinical outcomes of using EMD for periodontal regeneration which appear to be stable over the long term (Kalpidas and Ruben 2002; Venezia *et al.,* 2004; Esposito *et al.,* 2005; Tu *et al.,* 2008; Esposito *et al.*, 2009). Most recently it has been concluded that EMD is superior to control treatments for intrabony defects and as effective as resorbable membranes but superior to non-resorbable membranes for class II furcation defects (Koop *et al.,* 2012)

### *Combination treatments of guided tissue regeneration, EMD and bone grafts*

A number of studies have investigated whether there is any additive effect for the use of EMD when combined with bone grafts and or guided tissue membranes for the treatment of infrabony lesions (Zucchelli *et al.,* 2003; Gurinsky *et al.,* 2004; Sculean *et al.,* 2005; Bokan *et al.,* 2006; Kuru *et al.,* 2006; Hoidal *et al.,* 2008). Systematic reviews of the results of such studies have indicated that there is little evidence to support any significant additive effect of EMD in combination with other regenerative materials (Trombelli and Farina, 2008; Tu *et al.,* 2010).

### *Growth factors in periodontal regeneration*

Nearly all of the events associated with tissue repair and regeneration are regulated by polypeptide growth factors. Therefore it is logical to consider that these factors may be able to promote regeneration (Caffesse and Quinones, 2000; Giannobile *et al.,* 2010; Murakami, 2011; Stavropoulos and Wikesjo, 2012). A number of growth factors, both alone and in combination, have been studied for treatment of natural and experimentally induced periodontal defects in animal models (*Table 3*). Although there has been little uniformity among these studies in terms of study design, animal and periodontal defect model, types of growth factors and carrier vehicles, the results in general indicate that the application of various growth factors for periodontal regeneration produces favorable results (Reynolds and Aichelmann-Reddy, 2012; Darby and Morris, 2013). Despite a large body of evidence arising from both pre-clinical trials and randomized clinical trials, with the exception of Gem-21S®, a β-TCP/rhPDGF-BB combination (Osteohealth-Luitpold Pharmaceuticals, Shirley, NY), few of these growth factors have been developed into an everyday clinical practice product. This is largely due to a number of critical issues that still impede progress and need to be resolved. These include: (1) the complexity of the periodontium, which consists of four different tissues; (2) restricted understanding of the differentiation repertoire of the periodontal cells; (3) the exact target cells that are to be modulated by these factors; (4) the stability of the tissues that are to be formed under the influence of these factors; (5) the use of very high doses of bone morphogenetic proteins; (6) the ideal carrier has still not been found; and (7) the high costs that are associated with production of recombinant growth factors (Ripamonti and Petit, 2009; Bartold *et al.,* 2000). Thus, further investigation is needed to facilitate the clinical translation of the polypeptide growth factors and their delivery systems.

**Table 3.** Growth factors used for periodontal regeneration

| |
|---|
| Platelet-derived growth factor |
| Bone morphogenetic proteins |
| Transforming growth factor beta |
| Insulin-like growth factor |
| Fibroblast growth factor |

### *PepGen-15®*

The cell binding peptide P-15® (Dentsply Friadent, Mannheim, Germany) is a short polypeptide of 15 amino acids which mimics the cell binding domain of type I collagen combined with anorganic bovine bone-derived hydroxyapatite matrix. Its principal biological action is to enhance cell attachment of fibroblasts and osteoblasts, which may promote osteogenesis (Bhatnagar *et al.,* 1999). Several clinical studies (case series and a controlled monitored multicenter trial) investigating the efficacy of P-15® for periodontal regeneration have shown it to yield better clinical outcomes compared to the carrier alone (Yukna *et al.,* 2000; Yukna *et al.,* 2002). These clinical outcomes were found to be stable up to 3 years. However, no studies have made comparisons between P-15® and the gold standard of guided tissue regeneration or other regenerative procedures.

### *Platelet-rich plasma*

Platelet-rich plasma (PRP) is an autologous blood preparation enriched in growth factors such as transforming growth factor beta (TGF-β), vascular endothelial growth factor (VEGF), and platelet-derived growth factor (PDGF). Platelet-rich plasma has been used in various surgical fields, including maxillofacial and periodontal surgery with the expectation of enhancing bone and soft-tissue healing (Mehta and Watson, 2008; Foster *et al.,* 2009). To date the evidence for enhanced periodontal regenerative outcomes has been poor (Dori *et al.,* 2008; Kotsovilis *et al.,* 2010; Del Fabro *et al.,* 2011).

## Future technologies for periodontal regeneration

The emerging fields of personalized medicine and regenerative medicine are evolving quickly. Both are largely based on the concepts of tissue engineering, which is the science of reconstructing or mimicking natural processes through the use of synthetic polymer scaffolds with the expectation of tissue regeneration (Bartold *et al.,* 2000). The vision is that suitable cells, produced in large enough quantities through cell culture methods, together with appropriate bioscaffolds will be implanted into tissues and organs to produce fresh replacement cells to take over from lost or damaged cells and result in tissue regeneration.

### *Stem cells and tissue engineering*

An appealing approach to periodontal regeneration involves the use of cells, bioactive agents and biomaterials for therapeutics using tissue engineering principles (Bartold *et al.,* 2000). The concept of tissue engineering, taking into account the need for regenerative treatment of periodontal defects with an agent or procedure, requires that each functional stage of reconstruction be grounded in a biologically directed process. This biological technology, together with the emerging field of nanotechnology (the science of bioengineering at the molecular level to produce materials with hitherto unknown and unthought-of properties) will pave the future of periodontal regeneration. To this end there is considerable work being carried out with regards to the rational use of biodegradable scaffolds, informed use of instructive molecular messengers and the selection of specific cell phenotypes or even stem cells for periodontal regeneration.

Key factors in attaining successful periodontal regeneration are the correct recruitment of cells to the site and the production of a suitable extracellular matrix consistent with the periodontal tissues. As cell seeding to enhance regeneration of other tissues (skin, cartilage, bone, cardiovascular components, pancreas, etc.) has been used successfully (Persidis, 1999), it is seems logical that autologous periodontal ligament stem cells cultured within a suitable delivery scaffold, in conjunction with the growth and differentiation factors present in an autologous blood clot, will lead to new periodontal tissue attachment via a tissue engineering approach (Bartold *et al.,* 2006).

The discovery of periodontal ligament stem cells has opened a new vista for periodontal regeneration (Seo *et al.,* 2004). Many studies have now confirmed the presence of mesenchymal stem cell (MSC)-like cells in the periodontal ligament (Trubiani *et al.,* 2005; Nagatomo *et al.,* 2006; Gronthos *et al.,* 2006; Jo *et al.,* 2007; Techawattanawisal *et al.,* 2007). These cells have the characteristics of multipotency with an ability to differentiate into osteoblast, cementoblast or lipidogenic phenotypes. These cells are also able to survive cryo-freezing, which is of particular significance if these cells are to be "banked" for future use (Seo *et al.,* 2005).

Successful cell transplantation into periodontal defects and subsequent regeneration was first described over 20 years ago (van Dijk, 1991). Since then, a new field of periodontal cell transplantation opened up with encouraging results being reported. While the early investigations met with some success, overall the treatment outcomes were limited because of the heterogeneous nature of the cells used for such studies. More recently, the use of periodontal ligament stem cells for tissue engineering approaches to facilitate periodontal regeneration has emerged. To date most of the studies have been restricted to experimental animals, with only one report involving transplantation of periodontal ligament stem cells into human periodontal defects being published (Feng *et al.,* 2010; Hynes *et al.,* 2012).

Thus periodontal ligament stem cells can be used for regeneration of the periodontium in surgically created defects in both small and large animal models, albeit with limited success and in only a narrow field of application. A significant issue with these studies is that surgically created periodontal defects are very different from defects arising from periodontitis and thus any extrapolation of findings for stem cell regeneration in surgically created defects and what may happen in periodontitis needs to be made with caution. Another problem encountered with this approach is that very few of these stem cells attach to the surface of the alveolar bone and teeth. This led to the application of using cell sheet technology in conjunction with regenerative principles to deliver the regenerative potential of the periodontal ligament stem cells to the appropriate location (Iwata *et al.,* 2009; Washio *et al.,* 2010). This requires the identification and isolation of the cells required for periodontal regeneration and then growing these cells on a temperature-sensitive sheet in culture. Cell sheet construction involves the use of a temperature-sensitive polymer biomaterial, poly N-isopropylacrylamide (PIPA Am), in the cell culturing process. Once a mature cell sheet is formed, it is harvested by decreasing the temperature, which leads to detachment from the temperature-sensitive substrate. This allows harvesting of a complete sheet of cellular material with an intact extracellular matrix and cell-cell junctions, in an attempt to optimize any regenerative attempts. Recently a clinical study of periodontal regeneration using cell sheet technology in humans has commenced in Japan (Yoshida *et al.,* 2012). Following approval by the appropriate government regulatory bodies, autologous cell sheet transfers with autologous serum have been prepared using a standard operating procedure to ensure the quality of the transplant material. Following *in vitro* and *in vivo* testing, the cell sheets were prepared and approved for human clinical trials in January 2011. To date we still await the results of these trials, but on the basis of the preclinical trials the potential for this technology for periodontal regeneration is promising.

### *Gene and cell-based therapy*

Despite the emerging evidence that local application of growth factors may encourage periodontal regeneration, a number of issues remain which limit their efficacy. These include containment of the factor at the local site, limited controlled release of the bioactive peptides, and inactivation of the growth factor via locally produced proteinases. As a result, more refined techniques have been explored to improve growth factor delivery and release for periodontal regeneration. One such method is gene transfer, whereby genes for regeneration-promoting growth factors using plasmid and adenovirus gene delivery methods are used (Giannobile *et al.,* 1998; Zhu *et al.,* 2001)

Specifically, the use of adenoviral vectors encoding for growth factors such as platelet-derived growth factor and bone morphogenetic protein-7 has been investigated for use in periodontal regeneration (Anusaksathien *et al.,* 2003; Jin *et al.,* 2003; Jin *et al.,* 2004). These studies have shown that using such an approach there is sustained transgene expression for up to 10 days and enhanced bone and cementum regeneration at treated sites beyond this time period compared to the sites treated with control vectors (Jin *et al.,* 2003; Jin *et al.,* 2004). However, there is still a considerable amount of further work required before such an approach becomes a clinical reality. In particular, in order to maximize the duration and extent of gene expression, and ultimately to determine the success of gene transfer techniques in periodontal regeneration, the number of cells that are virally transduced to express specific genes needs to be optimized. Issues remain regarding the overall control of the process and how to both "turn on" and "turn off" the genes. In addition, research is required to assess the potential risks of the immunogenicity of viral recombination, which could significantly alter the success of gene transfer therapies for periodontal regeneration (Imperiale and Kochanek, 2004; Rios *et al.,* 2011).

## Summary and conclusions

Numerous techniques have been tried and tested to regenerate tissues lost to periodontal disease. While there has been some success to date, more work is required to move this to a reliable and clinically predictable procedure. Much of the future success for such treatments will rely largely on our understanding of the biology of both developmental and regenerative processes. Nonetheless, despite the noble goal of periodontal regeneration, the relevance of re-creation of a connective tissue attachment has been questioned. Since formation of a long junctional epithelial attachment to the tooth following a variety of periodontal treatment procedures has been shown to be no more susceptible to further breakdown than a non-diseased site, the question arises as to what purpose do we seek the ultimate outcome of periodontal regeneration? The answer lies in the "fact and fiction" of periodontal regeneration. There is no doubt that the regenerative procedures that have been developed can be shown to be biologically successful at the histological level. Furthermore, the results of periodontal regeneration (particularly guided tissue regeneration) have been stable over the long term (at least up to 10 years). However, the techniques currently under use which show the greatest promise (guided tissue regeneration and growth factors) are still clinically unpredictable because of their highly technique-sensitive nature. In addition, whether the slight clinical improvements offered by these procedures over routine open flap debridement procedures are of cost or patient benefit with regards to improved periodontal health and retention of teeth remains to be established.

The next phase in regenerative technologies will undoubtedly involve a deeper understanding of the molecular signaling (both intra- and extra-cellular) and cellular differentiation processes involved in the regenerative processes. So in answer to the question of whether periodontal regeneration is fact or fiction, the answer clearly is that it is both. However, with more work it will become established fact with little fiction and the desired clinical endpoint of predictable regeneration of the periodontal tissues damaged by inflammation to their original form and function will be achieved.

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