Chapter 8 Regenerative Periodontal Therapy

INTRODUCTION

The aim of periodontal regeneration is complete restoration of the attachment apparatus. Specific components such as periodontal ligament, cementum, and alveolar bone must contribute to this biological process for regeneration to occur. Periodontal ligament cells, osteoblasts, and cementoblasts must be in the regenerative site to achieve a clinically acceptable result. Regeneration, in contrast to conventional periodontal resective surgery, achieves pocket elimination/reduction and attachment gain by a biological process and not repair. Furthermore, pocket elimination procedures result in attachment loss that may cause root caries, sensitivity, and an esthetically compromised dentition. Meanwhile, regenerative surgical procedures alter factors during the wound healing process that shift repair to restoration of architecture. Ideally, a patient who suffered from periodontitis should have a periodontium comparable to a periodontally healthy individual following a regenerative treatment.

Regenerative periodontal procedures require biomaterials that lead to regeneration of the periodontal ligament (PDL) and new attachment. Earlier periodontal procedures for regeneration included bone and/or bone substitutes alone. A variety of barrier membranes to contain the graft material were later added to the procedure. In addition, graft materials or carrier materials (scaffold) are necessary depending on the purpose, e.g., space to be maintained at the site, and/or delivery of growth/regeneration factors. An ideal grafting material should deliver the regenerative signals in an optimal manner to stimulate a cellular response.

The specific predictability of periodontal regenerative procedures has focused on its indications. In general, the morphology of the bone defect limits the outcome. The procedures aim first to eliminate or reduce periodontal pockets, second to restore the lost alveolar process with regeneration of PDL and bone, and last to regenerate a functional attachment apparatus, ideally to periodontally healthy levels (Schallhorn, 1977). In general, indications for a regenerative procedure are deep intraosseous defects or cases in which osseous resective surgery is contraindicated because a substantial amount of supporting bone has been lost. In localized aggressive periodontitis, a regenerative approach should be considered first because osseous resective therapy can cause more harm. Regeneration minimizes post surgical clinical attachment loss.

Periodontal regeneration can be achieved by various techniques. These methods can be generally classified into grafting alone, guided tissue regeneration (graft and membrane), and growth factor stimulated regeneration. Grafting procedures alone can involve several material types including autogenous, allograft, xenograft, and alloplasts (Box 8.1). A variety of materials have the potential for periodontal stimulation for bone grafts. Materials commonly used for that purpose are osteogenic (bone graft material that has viable cells that can produce bone), osteoinductive (bone graft material that contains factors that may stimulate new bone growth), and osteoconductive (bone graft material that does not have any bone stimulatory factors in it but rather acts as a scaffold for bone growth).

Regeneration associated with grafting may result in long junctional epithelium because new PDL, cementum, and connective tissue (CT) attachment may not be established. However, acceptable clinical healing, including pocket reduction and attachment gain, may occur. Initial efforts to achieve guided tissue regeneration (GTR) aim to regenerate PDL and the rest of the periodontium by isolating the defect with barrier membranes and/or grafting to maintain space so that the regeneration can take place. Guided tissue regeneration is based on the exclusion of connective tissue and epithelium in favor of PDL regeneration, and following establishment of a new attachment. Thus, GTR is the purposeful selection of cell types that repopulate at the wound with the intention of directing the healing tissue composition. Barrier materials include natural absorbable polymers such as collagen (Types I, II, III, IV) and collagen and glucoseaminoglycan (GAG) copolymer; synthetic absorbable polymers such as polylactic acid and polyglycolic acid; fibrin; synthetic non-resorbable polymer polytetraflouroethylene (PTFE); synthetic ceramics such as calcium phosphate; and natural bone mineral.

Recently, the stimulation of periodontal regeneration with growth factors has become an effective and predictable technique. Based on biological enhancement of wound healing, these molecules produce a true histological regeneration. Growth/regeneration factors and differentiation factors such as enamel matrix proteins (amelogenin), polypeptide mitogens such as bone morphogenic protein-2(BMP-2), growth factors such as platelet derived growth factor (PDGF), and a combination of growth factors such as platelet rich plasma (PRP) are being used to stimulate regeneration. These molecules augment and/or stimulate the

BOX 8.1. Graft Material Types

Autogenous graft: Bone graft obtained from the same person either from intraoral or extraoral donor sites.

Allograft: Bone graft obtained from the same species as the recipient. A human bone graft is obtained from a donor (cadaver) and processed to decontaminate the graft material and make the particulate size adequate to use intraorally. Demineralized freeze-dried bone allograft (DFDBA) is the classical bone graft material used for GTR purposes.

Xenograft: Bone graft obtained from a different species than the recipient. An animal (typically cow) bone graft is obtained and processed to decontaminate the graft material and make the particulate size adequate to use intraorally.

Alloplasts: Synthetic or natural materials that can act as scaffold during bone regeneration. These materials must be bioinert and resorbable in the recipient site.

natural healing response and include stimulatory effects on angiogenesis, cellular differentiation, cellular proliferation, cellular ingrowth, and extracellular matrix biosynthesis, respectively. The stimulation by signaling molecules (growth factors) has improved the predictability in periodontal defects.

PERIODONTAL REGENERATIVE TECHNIQUES

The contemporary periodontal approach should eliminate the etiology and correct the defects by returning the periodontium to healthier levels. Periodontal regenerative procedures are necessary to achieve this goal (Figure 8.1). The advantage to periodontal regeneration not only includes attachment gain, but also provides benefits in areas with local or dental anatomical factors such as root concavities or where deep interproximal periodontal breakdown occurs. In addition, esthetics may be enhanced.

Guided Tissue Regeneration

Guided tissue regeneration is based on several key concepts such as new attachment, reattachment, regeneration, and repair. The term guided tissue regeneration was first used when a new connective tissue attachment was demonstrated with human histology by using a ePTFE membrane (nonresorbable) (Gottlow et al., 1986). Additional terminology includes new attachment (union of connective tissue or epithelium with root surface that has been deprived of its original attachment), reattachment (reunion of epithelium or connective tissue with root surfaces and bone such as occurs after incision or injury), regeneration (a histological term describing the reconstruction of an injured part), and repair (healing of a wound that is not fully restored to its original form and function).

Exclusion of epithelium is a critical component of barrier membrane regeneration. Epithelial cells migrate three to five times faster than PDL cells. As a result, the invasion by the epithelium does not allow PDL to regenerate. The first study showing that histological new attachment was possible used a Millipore filter to exclude epithelium (Nyman et al., 1982a,b), which showed that space creation by a membrane and/or graft is effective in periodontal regeneration. Cellular response investigations indicate that wound healing originates from the PDL (Aukhil et al., 1986). The peak mitotic activity of PDL occurs at approximately three weeks (Nyman, 1982). The importance of the PDL cell response in GTR is also critical to cementum formation, which allows PDL fibers to attach. Therefore, cementum formation is considered the rate-limiting step in periodontal regeneration (Caton et al., 1987).

Several factors are important in the outcome of guided tissue regeneration, including periodontal defect morphology, remaining periodontium, and practitioner skill. Guided tissue regeneration is technique-sensitive because the surgical area is narrow and soft tissue management is difficult. Meanwhile, flap design must consider primary closure of the surgical site to achieve full coverage of the regenerative membrane. An optimal amount of tissue must be retained in the flap design; typically, intrasulcular incisions are preferred. Releasing incisions also may be used as necessary (anatomy permitting) because access for thorough debridement of the surgical site from granulation tissue is important for success. Vertical incisions may also facilitate proper flap coverage of the membrane and graft material placement.

Following adequate flap elevation, the root surface must be debrided and deposits removed for new attachment. The consensus is that root conditioning with chemical agents is not recommended because no clear benefits have been established (Mariotti, 2003). Root surface mechanical preparation is essential in conjunction with hand instrumentation (curettes) or sonic or ultrasonic hand pieces.

Membrane adaptation around the tooth and onto the defect must be optimal to favor PDL growth without any significant epithelial downgrowth. Any significant irregularity of the bone that may affect the position of the membrane should be eliminated with conservative osteoplasty. As necessary, the membrane must be trimmed accordingly, adapted, and sutured around the tooth. Flap adaptation should be maximized around the tooth and onto the membrane for proper coverage. In addition, the flap should be passive onto the surgical site to prevent any dehiscence during the healing period. Suture material such as ePTFE or polyglycolic acid material should be chosen to close the site because these

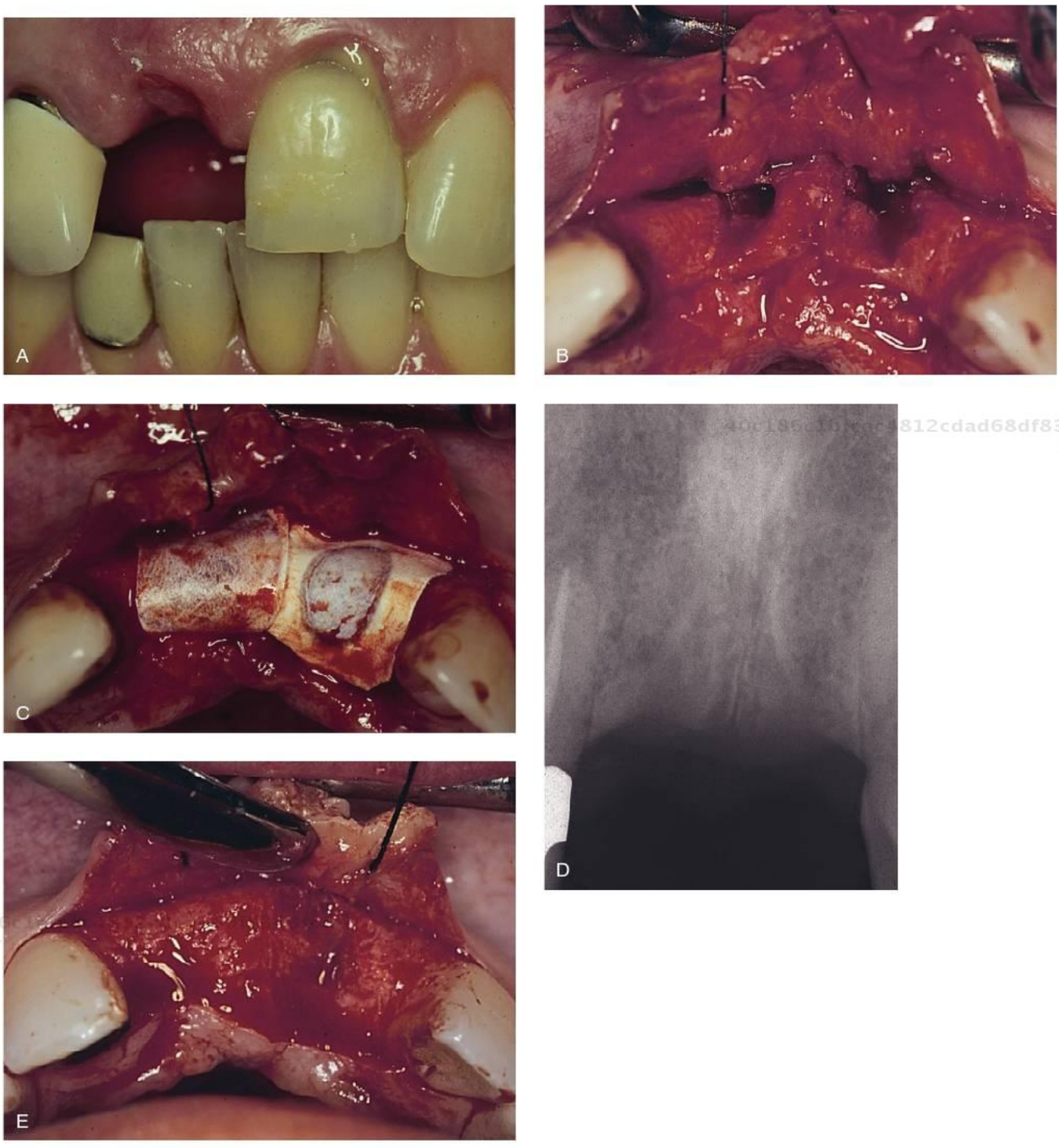
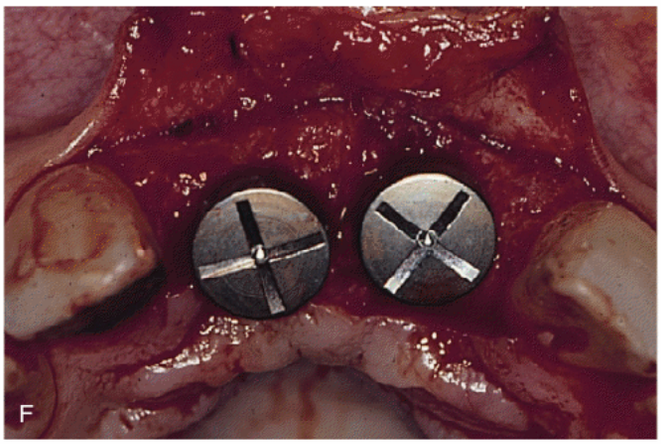
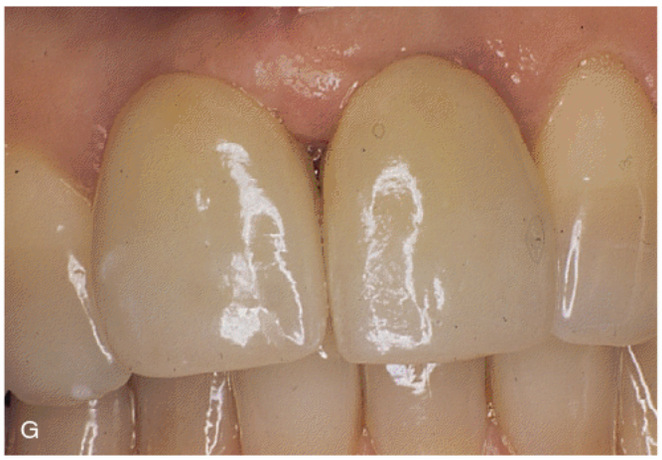


Figure 8.1. Extraction and ridge augmentation in the anterior maxilla with bone graft and resorbable membrane by guided tissue regeneration principles, implant placement, and final restoration. A, Fractured upper left central incisor (pus is noticeable on the gingival margin) and missing upper right central incisor. B, Upon flap reflection, extraction and degranulation were completed. Buccal plates for both sockets were entirely resorbed. C, Bone graft and resorbable membranes are in place. The membranes are critical for epithelial exclusion in favor of proper bone regrowth in the area. D, Radiographic evaluation prior to implant placement, approximately four months after bone grafting, reveals uniform bone formation. E, Complete clinical bone regeneration achieved approximately four months later. F, Implant placement at bone regenerated site to replace upper right central incisor and upper left central incisor. G, Final implant-supported fixed restorations for upper right central incisor and upper left central incisor with a desirable esthetic result.





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retain the least amount of plaque. Finally, a periodontal dressing may be used to secure the surgical area, especially from food impaction or trauma. Postoperative care is important and patients are typically prescribed an antibiotic, anti-inflammatory, and antibacterial rinse during the initial healing period.

Pontoriero et al. (1987, 1995) demonstrated in a series of studies that GTR is a predictable procedure for Class II furcations (Hamp classification) compared to open flap debridement. However, that predictability decreased significantly for Class III furcation-involved molars. For maxillary molars, buccal furcations are more predictable than the interproximal area. Meanwhile, a six month re-entry study showed that results were enhanced with GTR with a non-resorbable membrane and DFDBA, compared to GTR alone. The GTR and DFDBA combination produced more defect fill and pocket depth reduction and a greater gain in the clinical attachment level (Anderegg et al., 1991).

Depending on the number of bone wall defects, GTR showed 95% fill of three-wall, 82% fill of two-wall, and 39% fill of one-wall. Overall, a 73% defect fill was achieved (Cortellini et al., 1993a, b). In terms of defect fill, the areas of deepest probing depth demonstrated the greatest pocket depth (PD) reduction and clinical attachment level (CAL) gain (Selvig et al., 1993). Long-term results indicate that CAL gains were maintained four to five years following GTR (Gottlow et al., 1992). Good oral hygiene and patient compliance were found to be critical in long-term stability of GTR procedures (Cortellini et al., 1994).

Signaling Molecules in Periodontal Tissue Regeneration

There has been mounting evidence that the choice of graft material may play a significant role in altering the wound healing, from repair to regeneration. The analysis of autograft or allograft has found that signaling molecules contained in the material may stimulate regeneration. The molecular bases of these factors have been identified, characterized, and produced in large quantities. Several signaling molecules are currently being investigated with preclinical and clinical studies.

Enamel Matrix Proteins

Enamel matrix protein (EMP) or enamel matrix derivative (EMD) is a commercially available regenerative material. Amelogenin is the main constituent (95%) of this group of proteins; the remaining approximate 5% are other less characterized proteins. These proteins, especially amelogenin, are thought to be involved in acellular cementum, PDL, and alveolar bone development. The EMD that is currently available is isolated from porcine tooth buds (Hammarström, 1997). These porcine-derived proteins are very similar to proteins expressed during human enamelogenesis, but there are small genetic differences (Gestrelius et al., 1997a,b). In vitro studies with enamel proteins suggested that periodontal ligament cells' RNA expression is stimulated. Enamel proteins improved PDL cell function, particularly their effects on cell metabolism (Barkana et al., 2007). In addition, enamel proteins contributed to new blood vessel formation (Schlueter et al., 2007).

Osteoblast growth and differentiation, another critical component for periodontal regeneration, has been shown to be enhanced by EMD. In vitro studies indicate that EMD contributes to osteoblastic activity on human osteoblasts. In addition, it limits bone resorptive/osteoclastic activity. Moreover, important osteoblastic activity markers were enhanced at two and three weeks, an indication of continued biological activity (Galli et al., 2006).

Further evaluation of enamel matrix proteins for periodontal regeneration in a mouse model demonstrated that new cel-

lular cementum-like tissue formed along EMP-treated root slices (Song et al., 2007). Meanwhile, in order to understand the effects on soft tissues, human gingival fibroblast cells were treated with EMD and the cell cycle was assessed. The results suggested that EMD induced mitogenic activity for gingival fibroblasts, which may explain better soft tissue healing clinically (Zeldich et al., 2007).

Clinical investigations have demonstrated multiple benefits of the regeneration of periodontal support with EMD. The use of EMD in patients with lower molar Class II buccal furcation defects resulted in reduced horizontal probing at 14 months post-surgery as compared to those treated with traditional GTR (Hoffmann et al., 2006). Another trial evaluating contralateral randomized infrabony defects using EMD vs. GTR with resorbable membrane demonstrated similar improvements. These improvements in periodontal parameters were maintained up to eight years (Sculean et al., 2006). In addition numerous clinical case series have indicated positive results with EMD (Cortellini and Tonetti, 2007). A localized bone defect associated with a palatal groove was successfully treated with EMD. This particular treatment is limited to a case report with 8 mm clinical attachment gain and 2 mm of residual probing depth one year post surgically. However, it is viable evidence of the success for regenerative treatment in such complex lesions with EMD (Zuchelli et al., 2006).

Recombinant Human Platelet-derived Growth Factor

Recombinant human platelet-derived growth factor (rhPDGF) is a polypeptide growth factor which has been shown to stimulate periodontal regeneration. rhPDGF has specific effects on wound healing properties, including the regeneration of periodontium. rhPDGF is especially a strong mitogen for mesenchymal cells. Indeed, it has been shown that human osteoblastic cells produce PDGF. On a molecular basis, pdgf-a gene expression results in the PDGF protein. PDGF has PDGF-AA (acidic) and PDGF-BB (basic) variant forms. While human osteoblasts respond to PDGF-BB uniformly, there is an inconsistent response to PDGF-AA (Zhang et al., 1991).

Originally platelet-derived growth factor (PDGF) and insulin-like growth factor-I (IGF-I) were found, in combination, to synergistically upregulate DNA and protein synthesis in osteoblasts. Additional in vivo findings indicated that this effect would be achieved on soft tissue. Early studies suggested and evaluated the synergistic effects of PDGF and IGF-1 in periodontal defects in canine and human models with naturally occurring periodontitis. In preclinical models, rhPDGF-BB and rhIGF-1 in a carrier gel were applied on the root surfaces of periodontitis-affected teeth in conjunction with an open flap debridement procedure. Histological analysis of control carrier gel specimens indicated the presence of long junctional epithelium without any clear new attachment in two weeks. As expected, PDGF/IGF-1-treated sites demonstrated considerable new attachment as well as new bone highly populated with osteoblasts and new cementum. Significant osteoblast presence in PDGF-applied specimens was evidence of bone regeneration that perhaps would continue beyond two weeks.

Similarly, a carrier gel, with or without radioactively labeled rhPDGF-BB and rhIGF-1, was applied during periodontal surgery on beagles with naturally occurring periodontal disease. This experimental design made it possible to show the clearance rate of rhPDGF-BB and rhIGF-1 protein. The results revealed that the half-life of the molecules at the site of application was three hours for IGF-I and up to 4.2 hours for PDGF-BB. Almost all of the radioactively labeled protein was cleared after 96 hours, and two weeks later no radioactivity was detected. However, twice as much radioactive material was bound at the surgical site in the experimental group, which indicated that PDGF and IGF-1 bind on the target cells, which would trigger biological activity during periodontal regeneration. Histomorphometric analyses on two- and five-week specimens demonstrated five- to ten-fold increases in new bone and cementum as compared to controls (Lynch et al., 1991).

Meanwhile, the mitogenic effect of natural platelet-derived growth factor (nPDGF) on periodontal ligament fibroblast-like cells was investigated by using radioactive isotope incorporation in DNA. nPDGF stimulated approximately three times more DNA synthesis compared to the control and two times more compared to fibroblast growth factor (Blom et al., 1994). In vitro or laboratory findings are ultimately important for clinical practice to provide a proof of principle basis for biological activity. In that regard, in order for PDGF to optimally stimulate periodontal regeneration, the PDGF protein must bind its cellular receptor in periodontal tissues. Moreover, periodontal ligament cells may have the ability to differentiate into cementoblasts and/or osteoblasts with this stimulation.

Although PDGF-BB is well established as a key stimulator for periodontal regeneration in vitro and in vivo, further clinical studies were required to validate its effects on human periodontal defects. A split-mouth study was carried out with rhPDGF-BB and rhIGF-I in a carrier gel. Re-entry surgical procedures were performed to assess bone fill six to nine months following treatment. Results demonstrated that 2.08 mm of new vertical bone height gain and 42.3% osseous defect fill in rhPDGF-BB/rhIGF-1-treated defects occurred in comparison to 0.75 mm and 18.5% for the control sides (Howell et al., 1997).

In another study, purified recombinant human platelet-derived growth factor-BB alone (without IGF) with bone allograft as

the carrier was tested on interproximal intrabony defects and molar Class II furcation defects. Patients with teeth with advanced periodontal disease and poor prognosis that required extraction participated in the study. After the surgical debridement and notch placement on the root surfaces, osseous defects were filled with demineralized freeze-dried bone allograft (DFDBA) with one of three concentrations of rhPDGF-BB (0.5 mg/ml, 1 mg/ml, or 5 mg/ml). Bloc sections were taken, including the hopeless teeth and surrounding bone nine months following surgeries. Histomorphometric analyses were done in reference to the notch placed on the root surface. In the rhPDGF and allograft-treated defects, the vertical probing depth reductions as well as clinical attachment level gain for interproximal defects were approximately 6 mm, while radiographic bone fill was approximately 2 mm. Furcation defects treated with rhPDGF/allograft demonstrated a horizontal and vertical pocket depth reduction with clinical attachment level gain approximately of 3 mm. Moreover, histological evaluation indicated regeneration of a complete periodontal attachment apparatus with new cementum, PDL, and bone. Overall, this study demonstrated that rhPDGF-BB alone stimulates periodontal regeneration in both Class II furcations and interproximal intrabony defects (Nevins et al., 2003).

Further evaluations on clinical and histological response to rhPDGF-BB on Class II furcation defects were done with a similar study design. rhPDGF-BB stimulated periodontal regeneration, resulting in significant gains in horizontal and vertical probing depths and attachment levels. Similar to the previous study by the same investigators, histological specimens demonstrated that periodontal regeneration with new bone, cementum, and periodontal ligament used rhPDGF-BB with a xenograft (Camelo et al., 2003). Therefore, preliminary studies showed periodontal regeneration by returning attachment apparatus to healthier levels.

Following these findings, a prospective randomized, clinical study to determine the safety and effectiveness of rhPDGF-BB was completed. rhPDGF-BB and β-tricalcium phosphate (β-TCP) were used to treat advanced periodontal osseous defects and for comparison with the control. In this multi-center study, patients who required surgical treatment for a 4-mm or greater intrabony periodontal defect participated and were evaluated after six months. Experimental groups consisted of beta-TCP and either 0.3 mg/ml rhPDGF-BB or 1.0 mg/ml rhPDGF-BB, while the control consisted of β-TCP alone. The results demonstrated that rhPDGF-BB was safe for clinical use, since no adverse effects were detected. The clinical parameters that were used assessed PDGF-BB effects including clinical attachment levels, gingival recession, and radiographic linear bone growth and percent bone fill. The clinical attachment level gain was 3.8 mm for 0.3 mg/ml rhPDGF and was significantly higher compared to β-TCP alone at three months following the surgical procedures. However, at six months this difference was not statistically significant. The initial clinical attachment level gain acceleration with 0.3 mg/ml rhPDGF consequently caused a more significant clinical attachment level gain between baseline and six months compared to the control. Furthermore, at six months, rhPDGF-BB (0.3 mg/ml) treated sites also had significantly more linear bone gain (2.6 mm) and percent defect fill (57%) compared to 0.9 mm and 18% for the control group (Nevins et al., 2005).

The stability of the treatment during this clinical study was followed 18 or 24 months post surgically. All of the cases except one had their clinical attachment levels remaining intact. Moreover, linear bone gain and percent bone fill further improved compared to the six-month postsurgical results. The rhPDGF-BB-treated patients exhibited increased radiographic defect fill at 18 to 24 months post surgery compared to six months post surgery (McGuire et al., 2006).

In summary, this multi-center study demonstrated the safety of rhPDGF-BB for clinical treatment of periodontal defects. Treatment with rhPDGF-BB improved clinical attachment level gain at three months and maintained it up to 24 months. The results beyond six months showed further improvements in bone defect fill. the improvement of clinical attachment level is even more significant considering the multi-center nature of this study and the possible variability between both the operators and bone defects, despite standardization. Finally, expedited healing may be reflected in patient post surgical satisfaction and translate into easier patient management.

Predictable site development for dental implant placement and acceleration of healing at those sites are areas of interest to clinicians. In particular, the outcome of vertical ridge augmentation is difficult to predict due to a limited vascular and cellular supply. However, rhPDGF-BB stimulation may expedite angioneogenesis and cell migration in the grafted area.

A preclinical investigation evaluating the use of rhPDGF-BB with the presence or absence of a resorbable barrier membrane was investigated to assess its predictability for bone augmentation. Following dental extractions and surgical creation of ridge defects, bovine bone block with a collagen membrane, or bovine bone block infused with rhPDGF-BB, or bovine bone block infused with rhPDGF-BB with a collagen resorbable barrier membrane were used for vertical ridge augmentation. Histologic analysis indicated significant new bone formation and bone-to-implant contact in bone block and PDGF-BB-grafted sites. The absence of the barrier membrane positively contributed to the vertical bone regeneration, possibly due to better vascularization of the graft through the periosteum (Simion et al., 2006). This technique was recently assessed in humans. In a case report, the use of an rhPDGF-infused bone block was found to provide verti-

cal augmentation. However, this technique was surgically demanding and the bone block structure may have been less than ideal (Simion et al., 2008).

In summary, studies completed in vitro, in vivo, and in clinical levels show strong evidence regarding the potency of PDGF-BB in regard to periodontal regeneration (Figure 8.2). Indeed, PDGF-BB is considered to be a well established biologic/growth factor for periodontal regeneration. At this time there is limited but very promising evidence for guided bone regeneration.

Platelet Rich Plasma

The use of autogenous growth factors such as platelet rich plasma (PRP) have been investigated over the past decade. PRP is platelet aggregate obtained following blood centrifugation. It has been suggested that PRP has three times more growth factors such as TGF, PDGF, IGF, EGF compared to venous blood (Marx et al., 1998). Upon degranulation, platelet alpha-granules release growth factors that have stimulatory effects. Platelet counts and the presence of growth factors such as transforming growth factor-β (TGF-β) and PDGF are positively correlated in PRP concentration. Generally, PDGF is more than four times and TGF-β is more than three times more concentrated compared to venous blood. Both of these are well established osteogenic growth factors. However, the stimulation of osteoblasts by PRP was modest and only around 1.4 times more, compared to the control group (Okuda et al., 2003). The in vitro stimulation of human periodontal ligament cells, gingival fibroblasts, and keratinocytes by PRP resulted in variable effects of these cells. PRP stimulated periodontal ligament cells for collagen production four-fold in three days, and alkaline phosphatase, which indicates calcification in six days. PRP also decreased keratinocyte activity about 40% and did not significantly alter gingival fibroblasts (Annunziata et al., 2005). As mentioned above, PRP contains concentrated amounts of PDGF and TGF-β. Hence, human periodontal ligament cells were stimulated in vitro, with PRP obtained by centrifugation.

The creation of critical size rat or mouse calvaria defects is a classical animal model for evaluating bone graft effectiveness. PRP harvested from donor rats was applied in a collagen carrier matrix within rat calvaria defects. However, the PRP on rat calvaria model yielded variable results and was not significantly different than the control group (Pryor et al., 2005). In another investigation, PRP or platelet concentrate effects were evaluated on mini pig mandibles. Autogenous, xenogenous, and alloplastic bone grafts were also used in conjunction with PRP. No correlation between TGF and PDGF presence and platelet counts was present. Moreover, histomorphometric analysis in two, four, and eight weeks demonstrated no significant difference in bone formation with or without PRP presence (Jensen et al., 2005).

The application of PRP for human periodontal regeneration has produced a series of clinical case reports which indicate a positive but varied response. Periodontal regeneration with bovine bone in combination with PRP compared to open flap debridement in mandibular Class II defects demonstrated significant changes, especially for vertical and horizontal defect fill and clinical attachment level gains (Lekovic et al., 2003). Furthermore, GTR with bovine bone with or without PRP compared to GTR alone demonstrated modest effects for pocket depth reduction and clinical attachment level gain at six-month reentry results. This modest effect was not attributable to PRP since no significance was present between GTR with or without PRP. The combination with PRP compared to open flap debridement showed approximately 2 mm clinical attachment level gain (Camargo et al., 2003). Conversely in another investigation, PRP and bone graft with or without GTR showed a significant improvement for clinical attachment level. In this particular study, addition of GTR with a membrane did not make a significant difference (Lekovic et al., 2002). Furthermore, the application of PRP on intrabony defects in comparison to mineralized bone or bovine bone with membrane did not exhibit a significant advantage clinically after one year (Döri et al., 2007a,b).

Meanwhile, a lateral window maxillary sinus augmentation procedure may take between nine to 12 months before dental implant placement. Thus, addition of growth factors may be able to decrease this healing period. However, the addition of PRP to bone graft for lateral window maxillary sinus augmentation did not enhance the quality or the quantity of bone (Danesh-Meyer et al., 2001).

In summary, PRP was originally suggested to have three times more growth factors such as TGF, PDGF, IGF, EGF than venous blood, possibly leading to a modest increase in the bone density (about 25%) (Marx, 1998). However, the actual presence and concentration of cytokines in the PRP are not clear because there may be individual variations among patients. The state of platelets in the blood, whether they are stimulated for cytokine/growth factor expression at the time of blood withdrawal, are unknown. Moreover, isolation of PRP may directly affect the presence of cytokines simply because cytokines may not be expressed at the DNA level and consequently may not be synthesized at the protein level at any given time. Altogether, although PRP is conceptually a stimulator of a biological response, there may be several variables for clinicians to consider for predictable clinical results.

FUTURE OF PERIODONTAL REGENERATIVE THERAPY

Several concepts in regenerative therapy are in development. These include the application of recombinant human bone morphogenetic Protein-2 (rhBMP-2) and the more continuous application of signal molecules with gene therapy.

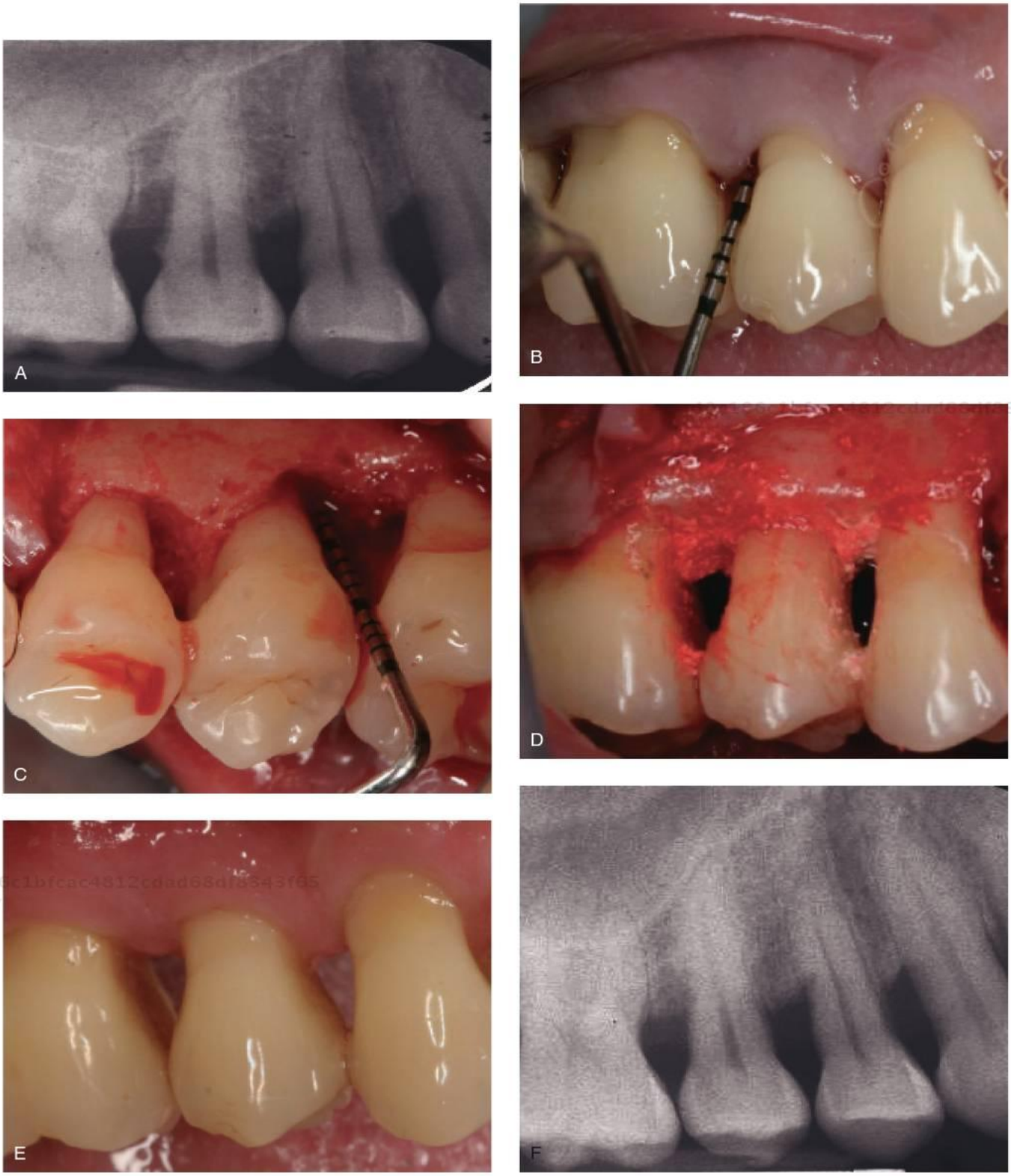


Figure 8.2. Guided tissue regeneration on upper right posterior sextant with recombinant human platelet-derived growth factor (rhPDGF). A, Periapical radiograph taken at the initial examination. Vertical bone defects as well as significant radiographic calculus are present in the area. B, Clinical picture after the completion of first phase of treatment. Probing depths up to 7 mm are present. However, the soft tissue profile is favorable for guided tissue regeneration. C, Clinical representation of bone defects following thorough debridement and degranulation during the GTR procedure. D, Application of rhPDGF with its carrier material (tricalcium phosphate) into the bone defects. E, Clinical healing eight months after the GTR procedure with probing depths not exceeding 3 mm. F, Radiographic healing eight months after the GTR procedure; bone fill is noticeable.

Bone morphogenetic proteins are involved in a wide variety of biologic activities, including embryogenesis. Except for BMP-1, which is an important factor for collagen synthesis (Uzel et al., 2001), the rest of the BMPs belong to the transforming growth factor family. In many biologic events different BMPs may work together. Among the numerous BMPs, BMP-2 and BMP-7 are known to stimulate periodontal regeneration.

The regeneration of the periodontal apparatus has been extensively investigated in preclinical models. rhBMP-2 periodontal regeneration in rodents was evaluated for early wound healing at 10 days and then 38 days. New bone formation was significantly increased 10 days after rhBMP-2 application on experimental periodontal wounds, as confirmed with histology. These exhibited new bone, cementum, and collagen fiber formation. Importantly, more cementum growth coronally was detected with rhBMP-2 application. Complete healing without any evidence of ankylosis occurred in all animals.

The application of rhBMP-2 for periodontal regeneration has been evaluated in canine and nonhuman primate studies. Various models, including naturally occurring or surgically induced, have been used to histologically assess the amount of regeneration. In general, new bone, cementum, and PDL were formed with the rhBMP-2. In addition, there were some indications of root resorption and anklyosis.

rhBMP-2 has been studied for its bone regenerative effects in preclinical and human studies. In a classical experimental model, calvaria were implanted with BMP/tricalcium phosphate (TCP) carrier in critical size defects in dogs. While rhBMP-2-stimulated sites demonstrated more than 90% new bone, the control group induced new bone formation below 10% (Urist et al., 1987). In animals, long bones were treated with rhBMP-2 in a collagen matrix carrier. Bone formation took place in a dose-related manner, while normal healing was observed for the control specimens (Yasko et al., 1992). Similar results were obtained in humans for long bone healing with rhBMP-2 (Johnson et al., 1988). Meanwhile, experiments on a calvaria model in Rhesus monkeys gave satisfying results, with critical size defect bone fill up to 100% with BMP-2 (Ferguson et al., 1987).

The clinical application of rhBMP-2 has involved bone and sinus augmentations (Figure 8.3). A randomized, masked, placebo-controlled, multi-center clinical study demonstrated that the novel combination of rhBMP-2 and a commonly used collagen sponge had a striking effect on de novo osseous formation for the placement of dental implants (Fiorellini et al., 2005). Two concentrations of rhBMP-2 were evaluated by using bioabsorbable collagen sponge vs. no treatment in a human buccal wall defect model following tooth extraction. Patients who required localized ridge augmentation for buccal wall defects and had more than 50% bone loss of extraction sockets of maxillary teeth participated in the study. The patients were randomly selected to receive 0.75 mg/ml or 1.50 mg/ml rhBMP-2/ACS, placebo (ACS alone), or no treatment. The efficacy of rhBMP-2 was assessed by evaluating the amount of bone induction and the adequacy of the alveolar bone volume to support an endosseous dental implant. Patients treated with 1.50 mg/ml rhBMP-2/ACS had significantly greater bone augmentation compared to the other groups. Moreover, the adequacy of bone for the placement of a dental implant was approximately twice as great in the rhBMP-2/ACS groups compared to those with no treatment or placebo. In addition, bone density and histology revealed no differences between newly induced and native bone. In a follow-up study, the efficacy of different rhBMP-2 concentrations in the anterior maxilla for the volume of bone regeneration was assessed with computer assisted tomography. These evaluations demonstrated a significant difference in bone formation between subjects treated with a concentration of 1.5 mg/mL rhBMP-2 (Bianchi et al., 2004).

The safety of rhBMP-2 at regenerative doses was assessed in the sinus augmentation indication. Either rhBMP-2 and absorbable collagen sponge at 0.75 mg/ml and 1.50 mg/ml concentrations or with conventional bone graft were used. Alveolar ridge height, width, and density were measured by computer assisted tomography scans taken prior to treatment, four months after treatment, and six months following implant loading. Alveolar ridge height at four months was similar in all three groups, at approximately 10 mm. The use of rhBMP-2/ACS was safe in terms of not causing any side effects or harm to patients (Boyne et al., 2005).

rhBMP-2 was also assessed for its use around dental implants, mainly aiming to understand its effect on osseointegration. For that purpose, perforated dental implants were placed in beagle dogs. Then, rhBMP-2 in a gel was applied onto these perforations and implant osteotomy. Histological sections were evaluated for the extent of new bone formation within the through-and-through perforations. Data indicated that significantly more bone formation occurred with rhBMP-2-treated sites when compared to the control gel alone (Fiorellini et al., 2001).

A similar study in dogs evaluated a synthetic bioabsorbable carrier and rhBMP-2 use in osseous defects around dental implants. Following the extractions of mandibular teeth, implants were placed in standardized circumferential bone defects with or without rhBMP-2. In addition, half of the implants were submerged with a non-resorbable, expanded polytetrafluoroethylene (ePTFE) membrane. Specimens were obtained at four or 12 weeks for histomorphometric analysis, which included percent new bone contact with implant, area of new bone, and percent defect fill. Although all implants were clinically and radiographically successful, the amount of

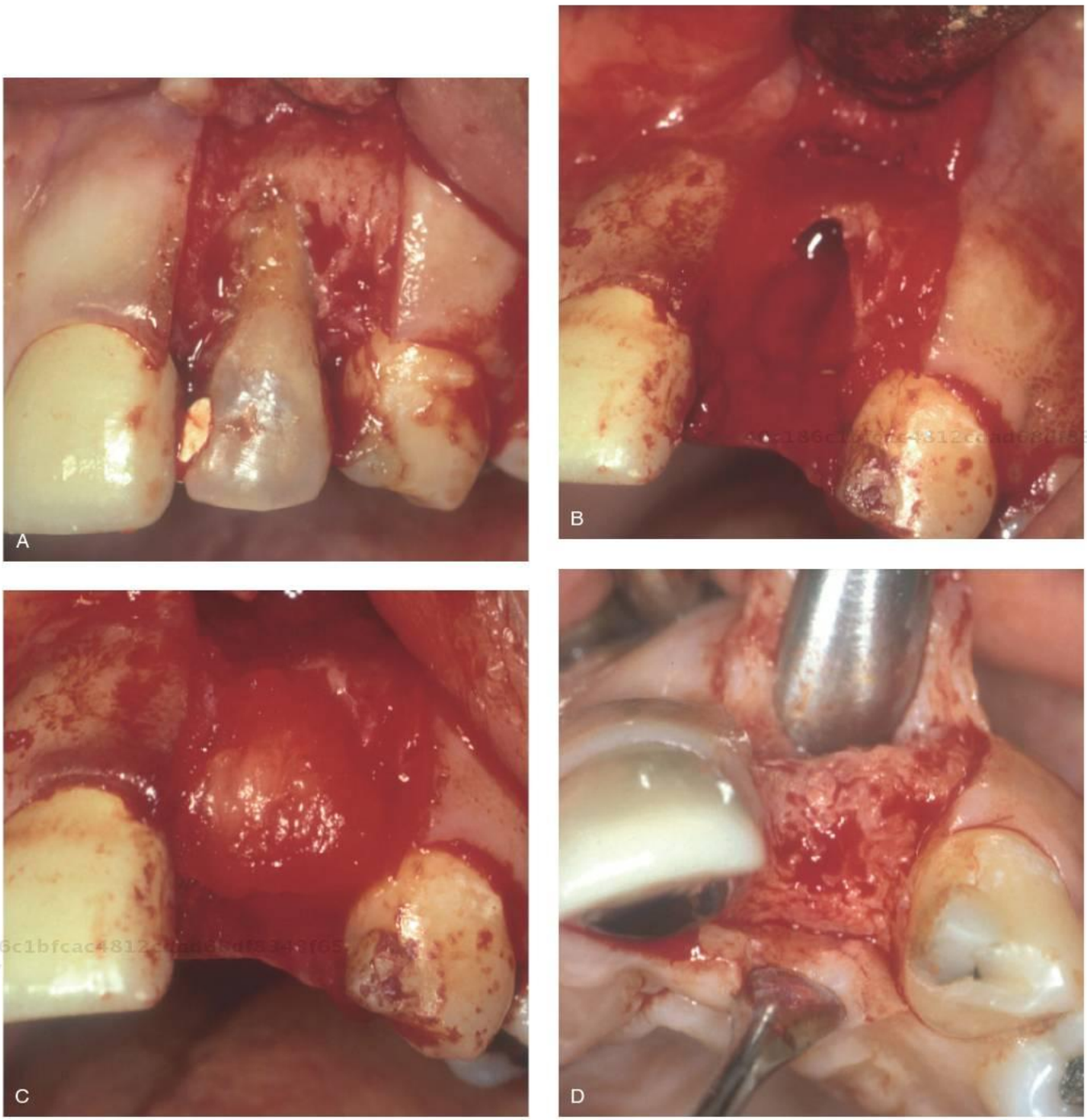


Figure 8.3. Guided tissue regeneration on an upper left lateral incisor site with recombinant human bone morphogenic protein-2 (rhBMP-2). A, The upper left lateral incisor is hopeless due to root caries. A significant dehiscence that contributed the etiology is present. B, The buccal plate is partially missing following extraction. C, rhBMP-2 with ACS (cellulose) carrier is used to augment the ridge. D, Complete bone regeneration is achieved three months after the guided bone regeneration with rhBMP-2.

new bone formation was dependent on rhBMP-2. The percent bone-implant contact was greater with rhBMP-2 in 12 weeks (Jones et al., 2006). However, membrane presence reduced bone formation.

Gene therapy approaches to bone tissue engineering have been widely explored. The maximum dose may be limited due to protein stability, half-life, and carrier properties. Even though topical application and/or delivery with carrier matrices are currently the best available clinical option, maintaining therapeutic protein levels at the surgical site may not always be possible. Gene therapy may become a viable option for continuous stimulation for periodontal regeneration.

Gene therapy is the alteration and manipulation of harvested target cells and redelivery of those cells into the target organ. A dose-dependent vector (adenovirus) was also used when critical size defects were on the femora of rats where the defects received different doses of plaque forming units of BMP-2 vector. Upon radiographic and histological evaluation, the high dose of vector bridged 100% of the femurs, while the medium-low dose did not exceed 25% (Betz et al., 2007). In addition, gene delivery using viral vectors may have some limitations for bone defects that need more time for healing. Thus, a condensing plasmid DNA with nonviral vectors such as polylactic/glycolic acid scaffolds were used for delivering plasmid DNA encoding for bone morphogenetic protein-4 into a cranial critical size defect for up to 15 weeks. Histomorphometric analysis revealed a significant 4.5-fold increase in total bone formation with a significant increase in both osteoid and mineralized tissue density for scaffolds incorporating condensed BMP-4 DNA in comparison to control (Huang et al., 2005). Neither of these more advanced techniques have been tried clinically. However, they certainly may have great use in larger sites such as lateral window maxillary sinus augmentation or vertical ridge augmentation as well as generalized periodontitis cases as indicated because these methods may significantly expedite healing.

In summary, BMP-2 is a potent mitogenic factor for periodontal and bone regeneration. The consensus is that animal and human studies show that bone regenerated with the application of BMP-2 resulted in bone density and histology no different than native bone. The application of rhBMP-2 for periodontal regeneration still requires human clinical evidence.

FUTURE REGENERATIVE THERAPY WITH rhPDGF

The topical administration of growth factors has been optimized for periodontal regeneration. Molecular biological techniques, such as gene therapy, have been used to demonstrate PDGF-BB effects on periodontal regeneration. PDGF-BB gene transfer was evaluated in rats for periodontal tissue regeneration in critical size alveolar bone defects. Several different gene combinations were used for PDGF-A and -B. Histomorphometric evaluations indicated that PDGF-B application stimulated cell proliferation compared to the control and PDGF-A. In addition, bone regeneration was four times greater for PDGF-B specimens compared to the other two groups. More importantly, gene expression remained active three weeks after PDGF-B application in periodontal defects (Zhu et al., 2001; Jin et al., 2004).

Although PDGF-A had low effects on periodontal regeneration for bone, its direct effects may be different on cementoblasts. Using a similar gene therapy technology, cementoblasts were altered with a PDGF-A gene. Gene-altered cementoblasts exhibited proliferation similar to that of continuous rhPDGF-AA protein application on native cementoblasts. RNA and protein analyses demonstrated significant presence for PDGF-A. This indicates gene delivery of PDGF-stimulated cementoblastic activity in addition to osteoblastic activity (Giannobile et al., 2001). The same group of researchers evaluated the effects of PDGF-A and PDGF-B gene transfer in human gingival fibroblasts in three-dimensional collagen lattices. An advantage to the 3D model is the possible quantification of defect fill. In this particular study, human gingival fibroblasts were altered with PDGF-A and PDGF-B genes. Findings of this study indicated that cell repopulation and defect fill was stimulated more than four times for the PDGF-B-gene-applied gingival fibroblasts, while PDGF-A and the controls exhibited similar diminished cellular activity (Anusaksathien et al., 2003).

While overall periodontal regeneration with rhPDGF-BB has been established, the clinical application of gene therapy may target specific cell types. In addition, the timing of protein expression may be critical to enhancing the clinical outcome. Gene therapy by turning on genes such as PDGF for sustained release will amplify the intensity and duration of periodontal regeneration. The findings for PDGF gene therapy confirm its potency for periodontal ligament cells and gingival fibroblasts as well as osteoblasts. As established for other systems, gene therapy is technically possible for periodontal regenerative treatment.

In summary, different treatment modalities have been introduced aimed at regenerating periodontal tissues lost secondary to the disease. The current regenerative options include the surgical placement of different bone grafts into the osseous defect sites, typically with a barrier membrane (Figure 8.4). Several biomaterials, agents, and growth factors for surgical procedures involving guided tissue regeneration have been developed. As reviewed, many studies support their clinical use. Certainly our goal as clinicians is to maximize the predictability of the prognosis of typically complex surgical procedures. Currently the additional benefits of growth factor proteins will enhance these patient-centered outcomes.

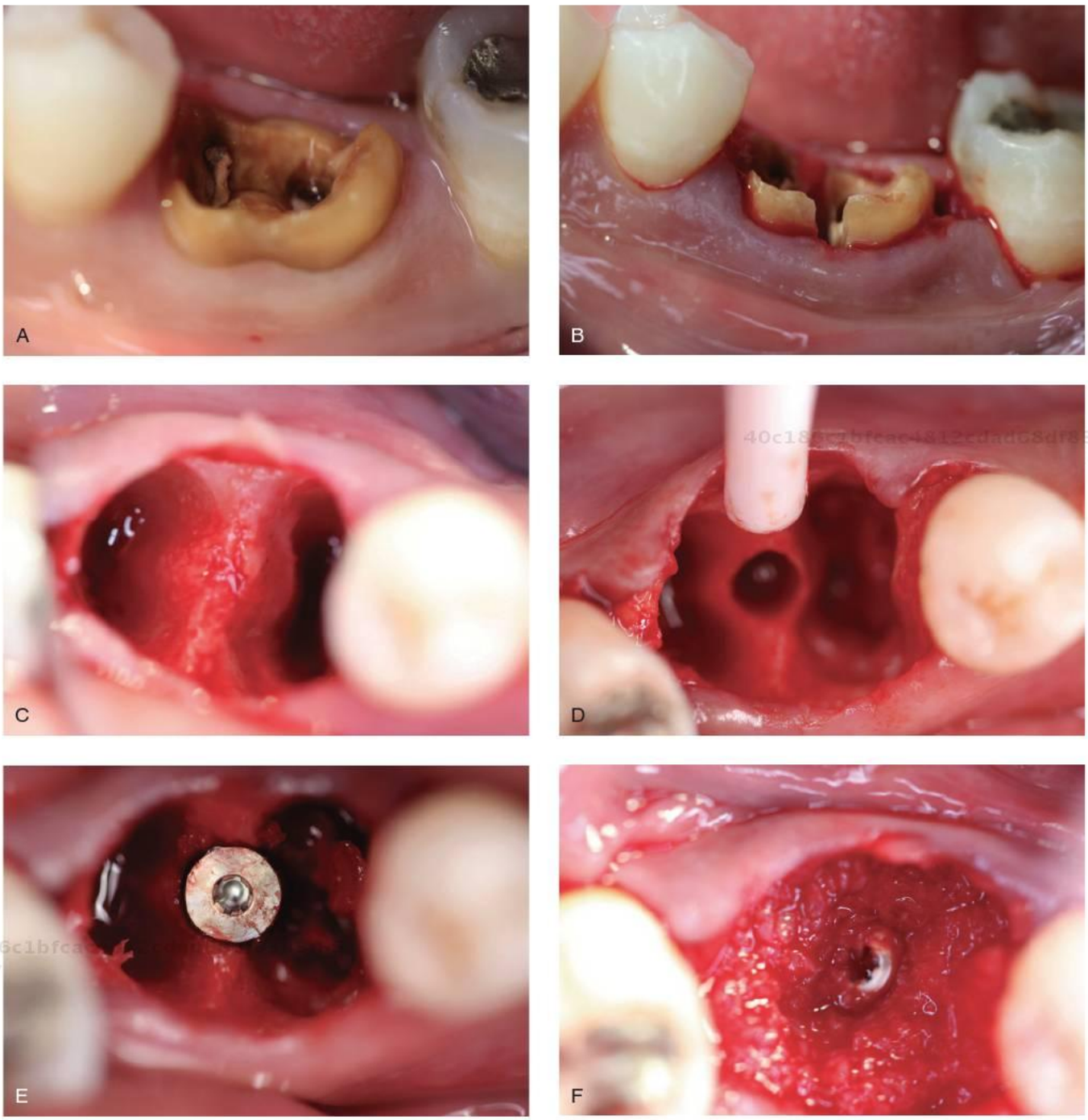
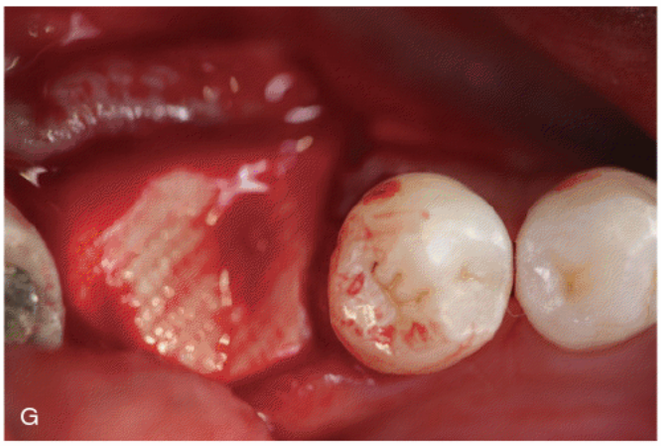


Figure 8.4. Guided tissue regeneration around the immediate molar implant placement. A, Lower left molar is hopeless due to the amount of recurrent decay around a PFM crown. Favorable intra-radicular bone and root convergence are present for an immediate implant placement. B, Sulcular incisions were made to preserve the maximal amount of soft tissue for the GTR procedure. The roots were sectioned for an atraumatic extraction. C, The extraction socket is favorable for an immediate implant placement and GTR. D, Osteotome prepared for a 4.5-mm diameter implant. An adequate amount of bone is present for initial stability and GTR. E, A 4.5 mm X 13 mm implant was placed in the prepared osteotome. F, Freeze-dried bone allograft (FDBA) was used to graft the remaining socket. G, A resorbable membrane was used to exclude epithelium and to contain the bone graft material. H, Primary closure of the soft tissue is essential for such a procedure. For that purpose, releasing incisions were done on the buccal aspect. In addition, simple interrupted and horizontal mattress suture techniques were used to minimize tension onto the flap and primary closure.



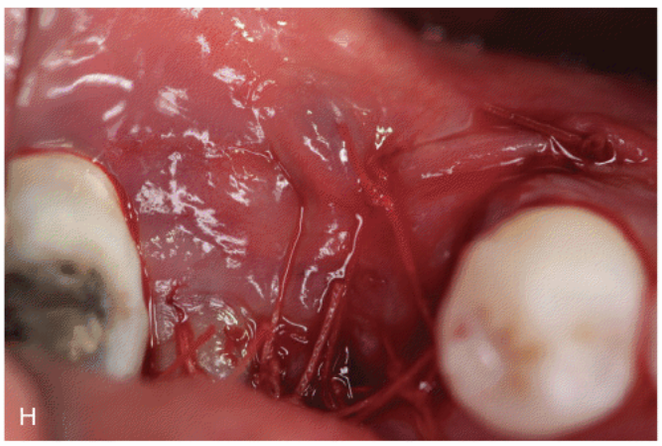


Figure 8.4. *Continued*

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