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25 Periodontal Regeneration and Reconstructive Surgery

Richard T. Kao

One of the initial objectives of periodontal therapy is infection management. Our understanding of the putative pathogenic periodontal microflora has altered our therapeutic approach from one of elimination of microbes to one of controlling pathogenic microorganisms and the immunoinflammatory response. Using treatments such as scaling and root planing, maintenance therapy, and antimicrobial therapy, our goal is to control the pathogenic microflora to prevent further periodontal destruction. Despite successful disease management, however, anatomic changes resulting from past disease activity often occur and must be corrected. Left untreated, these defects can provide a potential harbor for the reestablishment of pathogenic microflora. Thus, to facilitate long-term management of a healthy dentition, the periodontal defects must be eliminated. Therapeutic approaches for correcting these anatomic defects include procedures such as flap debridement/flap curettage, resective procedures, and periodontal regenerative therapy. Of these therapies, periodontal regeneration, or the complete restoration of the structure and function of damaged periodontal tissue, is the ideal goal. Over the last three decades several different techniques have been developed to achieve periodontal regeneration. Each technique has strengths and weaknesses. This chapter summarizes our current understanding of periodontal regeneration and examines how regenerative approaches toward correcting periodontal bony defects have changed over the years.

PERIODONTAL REGENERATION AND REPAIR

When the periodontium is damaged by inflammation or as a result of surgical treatment, the defect heals either through periodontal regeneration or repair.[1] In periodontal *regeneration*, healing occurs through the reconstitution of a new periodontium, which involves the formation of alveolar bone, functionally aligned

periodontal ligament (PDL), and new cementum. Alternatively, *repair* is healing by replacement with epithelium or connective tissue, or both, that matures into various nonfunctional types of scar tissue, termed new attachment. Histologically, patterns of repair include long junctional epithelium, new connective tissue adhesion, and/or ankylosis (Fig. 25-1).

On the cellular level, periodontal regeneration is a complex process requiring coordinated proliferation, differentiation, and development of various cell types to form the periodontal attachment apparatus. During tooth development, periodontal stem cells, originating from dental follicle cells, differentiate into cementum, PDL, and alveolar bone. Some stem cells remain in the PDL after tooth development. During periodontal wound healing, these stem cells, as well as those from the perivascular region of the alveolar bone, are stimulated to proliferate; migrate into the defects; and differentiate to form new cementoblasts, PDL fibroblasts, and osteoblasts.[2] This process of cell proliferation, differentiation, and maturation must occur in a synchronized fashion to form new alveolar bone, PDL, and cementum in a sequence such that these three individual tissues are integrated to function as a new periodontal supporting apparatus.

A number of periodontal regenerative approaches have been attempted with varying degrees of success, including the following:

- Root conditioning procedures: This strategy focuses on treatment with citric acid, tetracycline, or edetate disodium (EDTA) to demineralize the root surface. The conditioned root surface reportedly enhances the formation of new connective tissue attachment.

- Osteogenic vital bone grafts (autografts): Intraoral bone sites and iliac crests have been used as autogenous bone sources to correct intrabony and furcation defects.

- Osteoinductive nonvital bone grafts (allografts): Demineralized freeze-dried bone allografts (DFDBAs) and freeze-dried bone allografts (FDBAs) have been shown to induce bone formation.

- Osteoconductive materials: These materials include inert materials acting as biologic fillers (alloplastic materials), such as β tricalcium phosphate-hydroxyapatite, calcium ceramics-tricalcium phosphate, biocompatible composite polymers, and bioactive glass polymers; also included are organic materials such as coral and bone xenografts.

- Guided tissue regeneration (GTR): This process involves applying to the surgical wound site an occlusive barrier membrane that will prevent epithelial and connective tissue ingrowth. This enables stem cells from the PDL and perivascular tissue to repopulate the root area and differentiate into a new periodontal supporting apparatus. Occlusive barriers may be used either alone or in combination with a bone graft or alloplastic material.

• Biologic and biomimicry mediators: Purified biologic (enamel matrix derivative) or synthetic biomimicry agents (platelet-derived growth factors and bone morphogenetic proteins [BMPs]) have emerged as potential agents to enhance periodontal regeneration.

In this chapter, these various approaches to periodontal regeneration are reviewed. A comparative analysis of these various techniques is provided in Table 25-1. When analyzing this information, readers should consider the following questions:

1. What are the indicators for success with periodontal regeneration?

2. Are the clinical results superior to other therapeutic approaches (scaling/root planing, flap curettage, osseous resective surgery, and strategic extraction followed by implant placement)?

3. Which clinical approach, alone or in combination, will provide the best result?

4. Are the improved clinical results because of periodontal regeneration or repair?

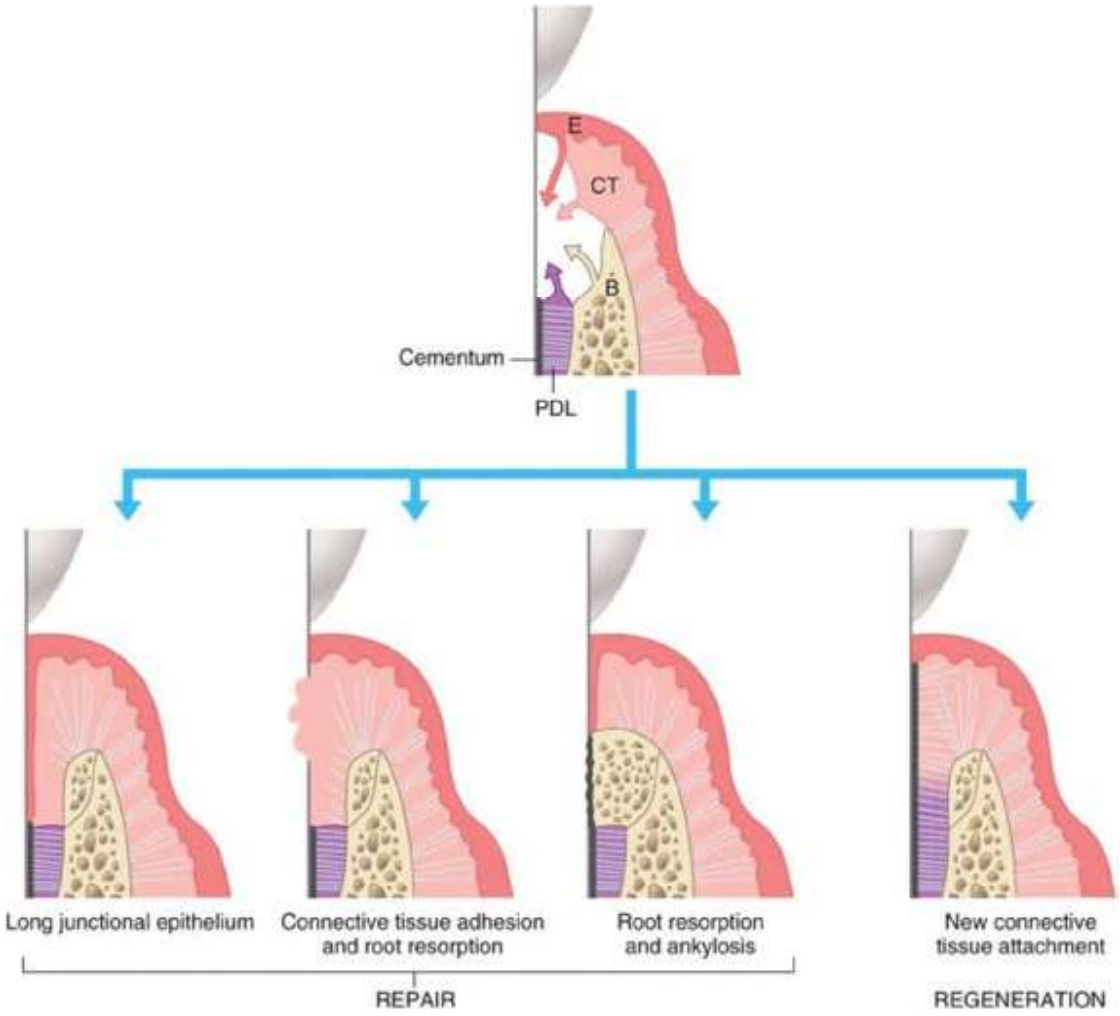
5. How stable are the clinical improvements? Are there 5-, 10-, 20-, or 30-year data to support the value of these techniques?

6. What are the clinical determinants that may influence the success of the periodontal regenerative approach?

7. Is patient compliance important?

8. How does one assimilate the information into a clinical decision tree for patient management?

Figure 25-1.



Possible healing patterns for a periodontal wound, which are dependent on the four possible cell types that predominate that wound site. The downgrowth of epithelial cells (*E*) results in a long junctional epithelium. The proliferation of connective tissue (*CT*) may result in connective tissue adhesion ± root resorption. With the predominance of bone cells (*B*), there may be root resorption, ankylosis (although this is relatively uncommon in humans when compared with animal models), or both. With the ingress of periodontal ligament (*PDL*) and perivascular cells from the bone, a regenerated periodontium with new cementum develops.

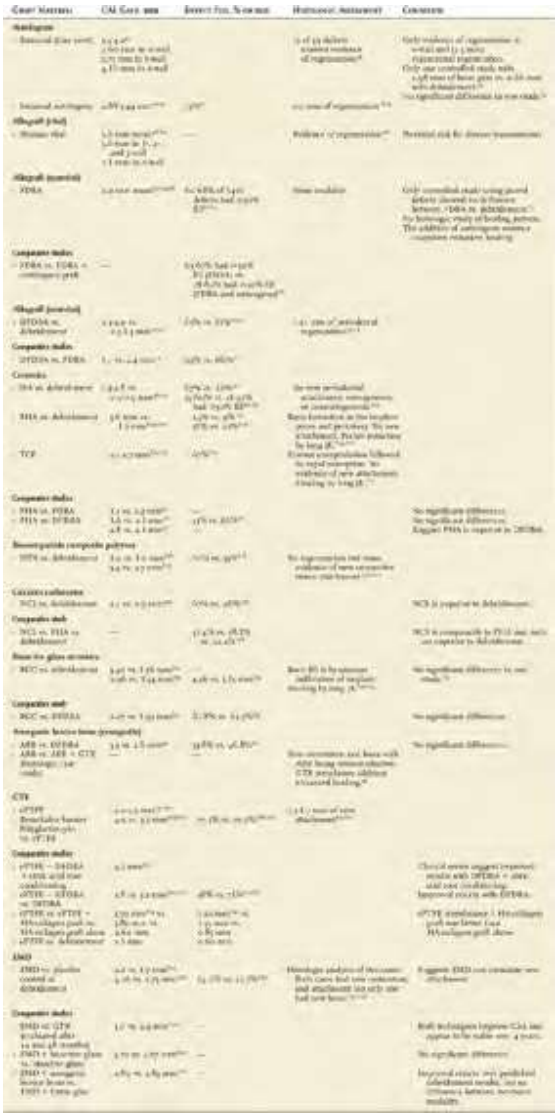
ASSESSMENT OF PERIODONTAL WOUND HEALING

The periodontal literature is replete with articles discussing various approaches for correcting periodontal defects. In these studies, a number of techniques are used for assessing periodontal wound healing. To properly evaluate each technique, it is important to understand the advantages and weaknesses associated with each.

Clinical assessment usually involves periodontal probing. Probing depth, the measurement from the gingival margin to the base of the sulcus, may vary depending on the amount of pressure applied to the probe and the degree of health versus inflammation present (see Chapter 8). With inflamed tissue the probe may penetrate past the initial connective tissue attachment to the root surface, whereas in periodontal health the probe may fall short of the connective tissue attachment.[3,4] Complicating this method of measurement is the amount of gingival recession that can result from past disease. Given these problems, changes in probing depth are of little value in assessing healing. The measurement that is usually used for clinical assessment is clinical attachment level—the distance from the cementoenamel junction to the base of the pocket. Most studies report changes in clinical attachment level. However, gains in clinical attachment level, although desired, do not necessarily imply that the new attachment is the result of actual regeneration (i.e., new bone, PDL, and cementum). The resolution of tissue inflammation, formation of a long junctional epithelial attachment, connective tissue attachment, and increased bone fill all result in clinical attachment level gain. In some studies, surgical stents have been used to ensure that the placement and angulation of the periodontal probe can be duplicated in subsequent dental visits, resulting in more accurate clinical attachment level measurements.

TABLE 25-1 Comparative Analysis of Regenerative Approaches

ABB, *anorganic bovine bone*; BGC, *bioactive glass ceramics*; CAL, *clinical attachment level*; DFDBA, *demineralized freeze-dried bone allograft*; EMD, *enamel matrix derivative*; ePTFE, *expanded polytetrafluoroethylene*; FDBA, *freeze-dried bone allograft*; GTR, *guided tissue regeneration*; HA, *hydroxyapatite*; HTR, *hard tissue replacement*; JE, *junctional epithelium*; PHA, *porous hydroxyapatite*; NCS, *natural coral skeleton*; TCP, *tricalcium phosphate*.



Bone fill is the only aspect of regeneration/healing that can be accurately assessed clinically. Formerly, bone fill measurements were performed by surgical reentry. This approach, however, is no longer recommended for routine clinical use because it requires an unnecessary second surgical procedure. Studies using nonsurgical means, such as bone probing or "sounding" performed with local anesthesia, have demonstrated accuracy in measuring changes in bone height equal to surgical reentry.[5,6] Although this can give an indication of how much bone has been produced, it is important to note that bone fill does not necessarily equate with regeneration. Bone fill is simply the formation of new bone within the periodontal defect; it does not describe how the tissue relates to the root surface. Histologic analysis has demonstrated that new bone fill may occur, and yet be separated from the root surface by formation of a long junctional epithelium or connective tissue adhesion, indicating periodontal repair.[7–9] In periodontal regeneration, functionally aligned PDL fibers are observed between newly formed bone and the root surface.

Standardized radiographic evaluation of bone regeneration provides qualitative evidence of bone fill, but yields little information in terms of the nature of the attachment and the density of the bone (Fig. 25-2). The amount of mineralization required to cause a detectable change in radiographic pattern makes this method of assessment less reliable than clinical probing techniques or surgical reentry.[10,11] With the advent of subtraction radiography, however, linear analysis has improved the radiographic assessment of bone fill.[12,13] This technique, used in conjunction with computer-assisted densitometric image analysis (CADIA), offers the greatest level of accuracy.[14,15]

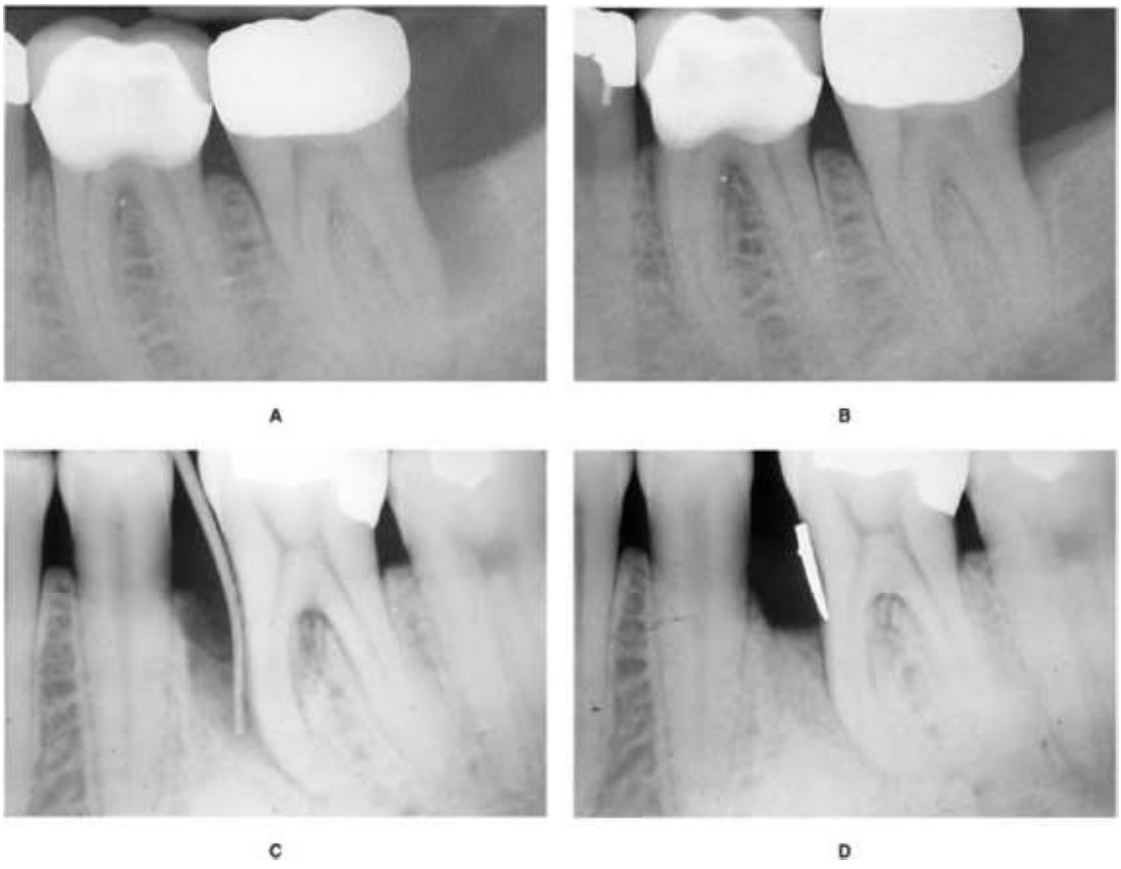
Histologic analysis is the only definitive method for determining whether the healing tissue was formed by repair or by regeneration (Fig. 25-3). This method provides an accurate assessment of the various components and their interrelationship in the newly formed periodontal attachment apparatus. In regeneration studies, reference notches are placed at the base of bony defects or at the apical extent of calculus deposits, and periodontal regeneration is considered to have occurred when the newly formed periodontium is coronal to the apical extent of the notches. Unfortunately, this approach cannot be used in human studies because it would be unethical to extract the treated tooth, especially when it responded positively to therapy. On rare circumstances, human histology is available if the tooth is to be extracted in conjunction with orthodontic or restorative therapy.

Several animal model systems can be used to study periodontal healing. Currently, the most widely used model systems include beagle dogs and nonhuman primates.[16] Because it is difficult to find an adequate number of naturally occurring periodontal osseous defects, the defects are either surgically produced or experimentally induced. Although the surgically produced defects may control the nature of the defect, they lack the chronic infectious properties observed with the naturally occurring disease process. The experimentally induced lesions have the chronic infectious properties, but it is difficult to control the type of osseous defects that result. Despite these weaknesses, these animal models permit the clinical and histologic study of the healing process.

The importance of histologic evaluation in confirming periodontal regeneration is exemplified in a classic primate study.[17] At the time of this study, the literature had described positive clinical outcomes from the modified Widman flap procedure, flap procedure with frozen autologous bone transplant, flap procedure with tricalcium phosphate (TCP) graft, and periodontal root planing and soft tissue curettage. When these four therapeutic approaches were examined histologically in the treatment of a monkey experimental periodontitis model system, all therapies resulted mostly in the formation of long junctional epithelium, a characteristic of repair, not regeneration. Although bone regeneration was detected in the intrabony defect, junctional epithelium was present between the newly formed bone and the root surface. These results suggested that apical migration of epithelial cells occurs more rapidly than colonization by other cell types. This study emphasized the importance of histologic confirmation of periodontal regeneration.

As the various approaches toward periodontal regeneration are reviewed, it is important to distinguish the type of defects being corrected and the results described. Improvement in clinical attachment level or bone fill radiopacity associated with the defect does not necessarily mean periodontal regeneration has occurred. The reconstitution of a new periodontium is a histologic determination that is difficult to obtain. Currently, the most widely used methods of evaluating whether a treatment modality can potentially result in periodontal regeneration are to provide histologic evaluation of periodontal regeneration in animal models when human biopsy materials are not available and to provide supportive clinical and radiographic data. The healing tissue often will contain areas of periodontal regeneration and areas of repair. Therefore, it is important to remember that, even in the presence of histologic evidence of regeneration, not all of the improvement in clinical attachment level is because of regeneration; some improvement is because of new attachment.

Figure 25-2.



A, Pretreatment radiograph demonstrating bony defect on the distal aspect of #18. **B**, Posttreatment radiographic appearance consistent with good bone fill. **C**, Pretreatment radiograph with gutta percha point noting bony defect. **D**, New osseous level with Hirschfield point postre-generative procedure. Although radiographs may indicate the presence of new bone, they may underestimate the amount of bone loss or gain, and they do not define the true nature of the newly

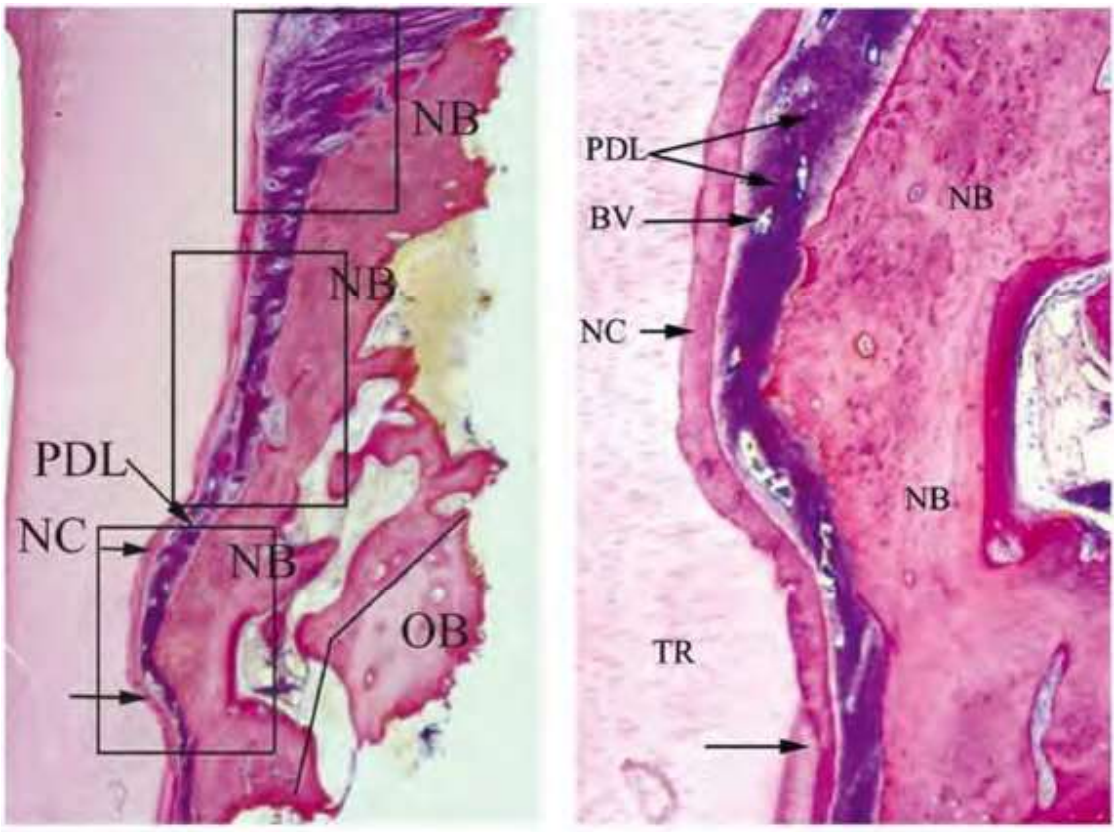
formed tissue. (**A** and **B**, Courtesy of Dr. J. Salzman, Larkspur, PA; private practice.)

THERAPEUTIC APPROACHES TOWARD PERIODONTAL REGENERATION

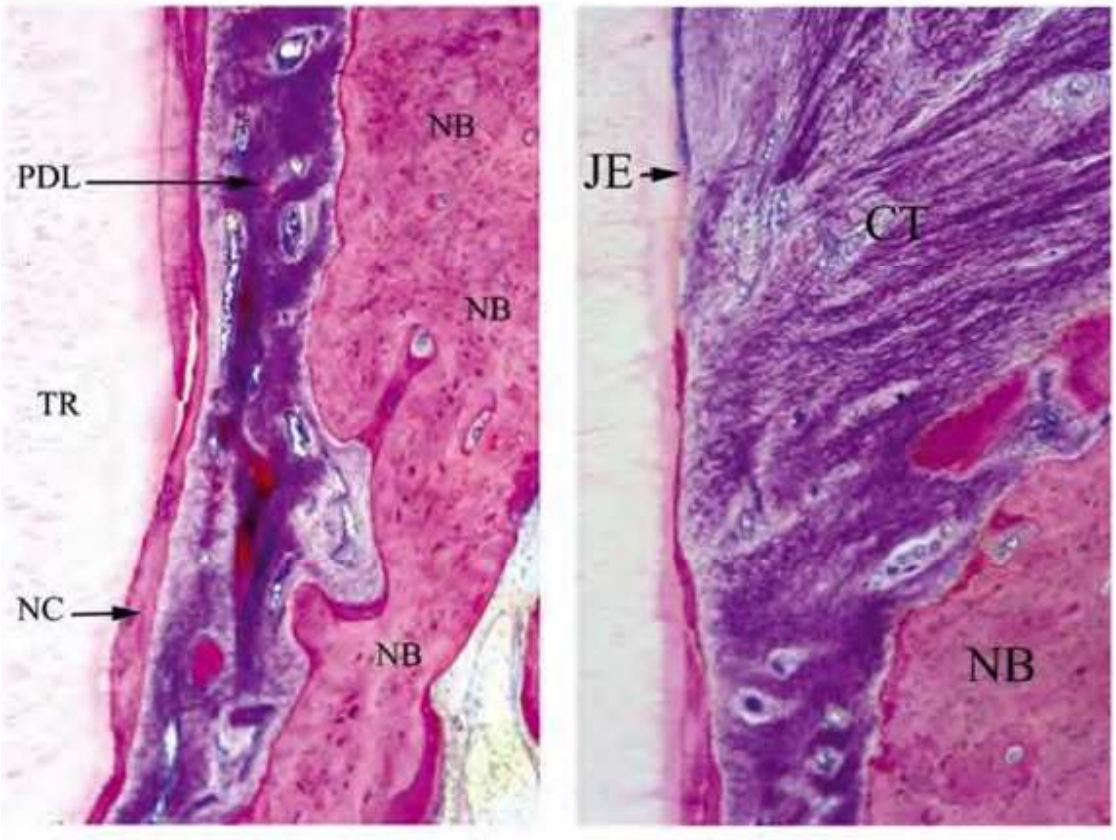
Root Conditioning Procedures

One approach toward improving periodontal healing is to clean and to enhance the root surface so that it is biologically compatible. Although scaling and root planing will remove bacterial endotoxins, early animal experiments indicated that demineralizing the root surface with citric acid at pH 1 for 2 to 3 minutes resulted in new connective tissue attachment and cementogenesis.[18–20] Histologically, the new connective tissue attachment appeared as perpendicularly arranged collagenous fibers, which were continuous with the newly formed cementum. Several studies analyzed the healing sequence of the citric acid-treated root surface. With treatment, the smear layer (a surface layer of protein and debris) was removed and the root surface was demineralized to expose a 2- to 15-µm zone of thick collagenous fibrils anchored to a root surface with opened dentinal tubules.[19,20] After 1 to 3 days, a fibrin linkage was present between the PDL and root surface. By 21 days, the fibrin network appeared to be well attached to the root surface. This fibrin linkage has been shown to impede the apical migration of epithelium and result in a rapid ingress of cells, which putatively will develop the new connective tissue attachment.[21–24] Interpretations of histologic studies suggest that the formation of new attachment is the result of interdigitation between newly synthesized collagen fibrils and established collagenous fibrils of the cementum or dentin. The histologic findings of citric acid treatment have been confirmed in human studies.[25–29]

Figure 25-3.



A B



C D

Photomicrographs of a clinical case of periodontal regeneration 9 months after the application of recombinant human platelet-derted area (**A**); a detailed view of the notched root surface (**B**) and the newly regenerated periodontium coronal to this landmark (**C**); and the coronal aspect of the regenerated periodontium in relation to the junctional epithelium (**D**). BV, blood vessel; CT, connective tissue; JE, junctional epithelium; NB, new bone; NC, new cementum; OB, old bone; PDL, periodontal ligament; TR, root trunk. (Courtesy of Dr. S. Lynch, Biomimetic Pharmaceuticals, Inc.; Franklin TN.)

The positive attributes of citric acid treatment are supported by two clinical studies.[30,31] In a split-mouth design to evaluate the effect of citric acid treatment after replacement flap surgery, citric acid at pH 1 was applied to the root surface for 3 to 5 minutes. An average clinical attachment level gain of 2.1 mm was observed for the citric acid-treated side as compared with a 1.5 mm gain for the control side. In a similar study evaluating citric acid use with root surfaces associated with intrabony defects, the gain in clinical attachment level was 2.0 mm, whereas the nonacid-treated control had a clinical attachment level gain of only 1.2 mm. Approximately 73% of the acid-treated teeth gained 2 mm or more in clinical attachment level. Contrary to these studies, others have not been able to reproduce histologic evidence of new attachment[32–34] or beneficial clinical results.[35–38]

Root conditioning with tetracycline and EDTA also has been advocated. The suggested benefit is that it may produce a histologic phenomenon similar to citric acid treatment without inducing pulpal or epithelial injuries.[39,40] Histologic information on these agents, however, is limited. There are other indications that may warrant the use of tetracycline. Tetracycline has been shown to bind to dentin with the maximum binding occurring when tetracycline is applied at 50 mg/ml or greater. The bound tetracycline is released and serves as a local antimicrobial delivery vehicle for up to 14 days.[41,42] In addition, tetracycline-treated dental slabs have been shown in cell culture experiments to bind fibronectin, a cell adhesion protein that mediates cell attachment and migration of mesenchymal cells. The presence of fibronectin permits increased cell adhesion and colonization. Conversely, tetracycline reverses the binding of laminin, an epithelial cell attachment protein.[43] These studies suggest that tetracycline treatment preferentially permits the colonization and migration of fibroblasts over epithelial cells.

Two clinical studies have disputed the efficacy of tetracycline root conditioning. In the first study, diseased root surfaces underwent root planing and were treated with tetracycline burnished for 3 minutes, tetracycline burnished with an application of exogenous fibronectin, or no treatment. No new attachment was present in any of the groups. The tetracycline treatment resulted in a statistically significant improvement in clinical attachment level, but the difference was clinically insignificant.[44] When tetracycline treatment was used in conjunction with GTR, there were no clinical improvements observed when compared with control.[45] A comprehensive, systematic review of all the evidence for root conditioning with citric acid, tetracycline, and EDTA in humans found no statistically or clinically significant benefit to use of any of these agents.[46] Despite the lack of evidence,

some practitioners continue to perform root conditioning in certain cases. There appear to be no adverse effects of such treatment.

Bone Grafts and Grafting Materials

The classical approach to periodontal regeneration in the last 30 years has been the use of bone grafts or bone substitutes in repairing periodontal defects (Box 25-1). Grafts are generally classified according to their original source as follows:

Autograft: Tissue transferred from one position to another within the same individual.

Allograft: Tissue transferred from one individual to another genetically dissimilar individual of the same species.

Xenograft: Tissue transferred from one species to another species.

Alloplast: A synthetic graft or inert foreign body implanted into tissue.

Box 25-1 Bone Grafts and Bone Substitutes Used in the Correction of Periodontal Defects

Bone-Derived Material

Vital Bone Graft

- *Autograft*

Oral

Osseous coagulum

Bone blend

Bone harvested from extraction site, tuberosity, edentulous ridge

Extraoral

Iliac crest

- *Allograft*

Cryopreserved bone

Fresh bone from iliac crest

Nonvital Bone Graft

- *Allografts (human bone)*

  Freeze-dried bone allograft

  Demineralized freeze-dried bone allograft

- *Xenograft*

  Anorganic bovine bone

Nonosseous Material

Organic

- *Dentin*
- *Cementum*
- *Coral*

Anorganic (alloplasts)

- *Calcium sulfate (plaster of Paris)*
- *Calcium phosphate-hydroxyapatite*
- *Calcium ceramics*
- *Bioactive glass polymers*

Early clinical series reported that bone regeneration was enhanced by the use of cancellous bone autografts from the iliac crest. These fresh autografts are *osteogenic*, that is, vital cells present within the grafted material are capable of forming new bone. Although this method proved clinically successful, the necessity of a secondary surgical harvest using an extraoral site (the hip) and surgical complications of ankylosis and root resorption of the treated tooth or teeth made this approach less popular. Therefore, during the last decade, FDBA and DFDBA have become the materials of choice. These materials are widely available and may induce new bone formation. However, studies have questioned the bone inducing properties of bone allografts, suggesting that this potential may vary depending on the bone bank or batch within the bank used, processing procedures, and donor characteristics. Alternatively, a variety of xenograft and alloplastic grafting materials have become available for use in periodontal regeneration and repair. This section reviews the clinical and histologic results after the use of these materials.

Bone grafts and bone substitutes used in regenerative therapy are derived from bone or nonosseous materials. Correction of osseous aspects of the periodontal defect occurs by osteoinduction or osteoconduction. A graft material is *osteoinductive* when it can induce bone formation. This implies that the material is able to recruit

undifferentiated mesenchymal cells, be mitogenic for preosteoblasts, and induce differentiation of these cells into bone-forming osteoblastic cells. A material is *osteoconductive* when its structure and chemical composition facilitate new bone formation from existing bone. Osteoconductive materials generally act as scaffolding on which new bone forms. This often results in the amalgamation of the material into the newly formed bone mass.

Osteogenic autogenous bone grafts (autografts)

Iliac bone and marrow autografts have proven to be the most predictable graft materials for bone growth. However, because of the necessity of harvesting from a secondary surgical site and the possible morbidity associated with these procedures, they are no longer popular. In an early study, Schallhorn and colleagues[47] treated 182 osseous defects ranging from 3.3 to 4.2 mm in 52 patients with iliac graft. The resultant mean bone fill was 2.6 mm in "zero wall or no wall" defects, 3.75 mm in one-wall defects, and 4.16 mm in two-wall defects. Approximately 87% of the Class II furcations had complete fill. Histologically, new bone, cementum, and functionally oriented PDL were observed.[48] Complications associated with the use of fresh iliac bone and marrow included a high rate of root resorption and anklyosis.[49] These complications were later shown to be minimized by either freezing the bone graft in a storage medium or adding autologous intraoral bone to the harvested iliac crest bone graft mixture. To date, iliac bone and marrow have the most osteogenic and regenerative potential, and are one of two graft materials with the reported ability to regenerate periodontium horizontally or with "zero wall" defects, meaning actual crestal apposition of new bone.

Intraoral autogenous bone grafts have been harvested from various intraoral sites including edentulous ridges, the maxillary tuberosity, 8- to 12-week postextraction healing sites, and tori or exostoses. Three clinical case series described the use of intraoral cortical-cancellous grafts, which resulted in bone fill of 2.88 to 3.44 mm in 373 defects.[47,50,51] One controlled study of 37 paired defects demonstrated 2.98 mm of bone gain when autogenous intraoral bone grafts were used, as compared with 0.66 mm for debrided controls that received no grafts.[52] With the exception of furcations and crestal defects, intraoral bone grafts were comparable to iliac grafts. Contrary to these findings, two controlled studies indicated no significant differences in bone gain.[53,54] These conflicting reports may be because of site morphology and donor tissue. Studies have indicated that the degree of success and increased amount of bone fill are related to the increased number of osseous walls associated with the defect. The source of intraoral bone also is important. When bone is predominantly cortical in nature, it has little osteogenic potential. Cancellous bone, which contains hematopoietic marrow, such as red bone marrow from the maxillary tuberosity or from healing bone sockets 8 to 12 weeks after extraction, provides better osteogenic potential. According to two reports,[48,55] when intraoral autogenous bone is used in a composite graft with FDBA, regeneration is enhanced as compared with FDBA

alone (78%–80% vs. 63%–67% of defects exhibiting greater than 50% bone fill). Histologically, several studies[48,55] and case reports[56–59] have shown that intraoral autogenous bone is able to form new attachment.

These clinical studies suggest that autografts can effectively enhance bone fill by an average of 3 to 4 mm. Currently, this is considered the "gold standard" for periodontal graft material.

Osteoinductive nonvital bone grafts (allografts)

In a series of animal experiments and clinical case series, Schallhorn and Hiatt[48,60] reported that when allografts of iliac bone and marrow were used, the results were similar to autogenous iliac grafts with mean bone gain of 3.6 mm in one-, two-, and three-walled defects; 2.1 mm vertical increase in "zero wall" defects (crestal apposition in cases with horizontal bone loss patterns); and 3.3 mm bone gain in furcation defects. Notably, this is the only other graft material with reported ability to correct "zero wall" defects. However, despite these encouraging results, the risk for disease transmission from the donor to the graft recipient has eliminated the potential use of frozen allografts in periodontics. Allografts used in periodontics are primarily in two forms: FDBA and DFDBA. These allografts are processed in such a way as to minimize the risk for disease transmission.

In four uncontrolled studies, FDBA has been shown to be effective in correcting osseous defects. These studies involved a total of 1401 defects, and results consistently indicated that 60% to 68% of the defects had 50% or more bone fill on reentry.[61–64] Osseous regeneration was least pronounced in furcation defects.

FDBA used alone and augmented with other graft materials also has been tested and compared with other grafting procedures. Studies using FDBA augmented with autogenous bone found that an additional 11% to 17% of these defects had 50% or more fill when compared with defects treated with FDBA alone.[62,64] A comparison of FDBA with granular porous hydroxyapatite (PHA) indicated that FDBA was superior, with 2.1 mm of bone fill compared with 1.3 mm for granular PHA.[65]

Currently, there is no histologic evidence of periodontal regeneration after FDBA grafting procedures. Although clinical reports of osseous fill are impressive, with approximately 60% of the defects having 50% or more fill and the mean bone fill approximately 2 mm, the only controlled study to date showed no difference between the use of FDBA versus debridement in a small number of paired defects.[66]

Animal studies by Urist and colleagues[67,68] and other studies[69] have shown that demineralization of cortical bone allografts will improve the osteogenic potential by exposing BMPs, an inductive factor known to increase bone formation. Tissue banks have used modifications of this protocol to process DFDBAs. In human histologic studies, Bowers and colleagues[70,71] demonstrated that the mean new

attachment formation for 32 defects was 1.21 mm when DFDBA was used, whereas no new attachment was observed in 25 debrided defects that received no grafts. Clinical studies have shown that using DFDBA results in more bone fill as compared with controls in which only debridement is performed (2.3–2.9 mm vs. 0.3–1.3 mm and 65% vs. 11–37% bone fill).[72–74] In clinical comparison studies, DFDBA has been shown to be comparable to FDBA[75] and comparable[76] or inferior to PHA.[77]

Recent studies have focused on three issues: (1) Are DFDBA grafts osteoinductive?; (2) Can the osteoinductive potential of DFDBA be improved?; and (3) What is the long-term outcome of DFDBA-treated sites?

Are demineralized freeze-dried bone allografts osteoinductive? The clinical premise for using DFDBA was based on Urist's studies[67,68] that suggest demineralization of FDBA will make BMP accessible for osteoinduction. Although BMPs are genetically highly conserved, FDBA and DFDBA are immunogenic between species.[78] To eliminate the immunogenicity issue, Becker and colleagues[79] implanted human BMP preparations and DFDBA from four commercial bone banks into muscle pouches of athymic mice, which were genetically immunosuppressed. Histologically, commercial DFDBA induced minimal amounts of new appositional bone (7.5–21.6%). However, Urist's partially purified human BMP preparations resulted in 96% of the field filled with new appositional bone.[80] The discrepancy between Urist's preparation and commercial DFDBA preparations may be because of the modification of the bone-processing protocol used by the bone bank to minimize risk for infection. Furthermore, denaturation of BMPs may occur during large batch processing of commercially available DFDBA.

To address the issue of whether laboratory-prepared DFDBA is different from commercially available DFDBA, Shigeyama and colleagues [81] compared the protein extracts from these DFDBA preparations in terms of their effects on early events of bone formation—for example, cell recruitment, attachment, and proliferation. The laboratory preparation was more mitogenic and resulted in a faster rate of cell proliferation than the extracts from commercial DFDBA. All extracts enhanced cell attachment, whereas no extracts were effective in cell recruitment and chemotaxis. When matrix proteins were analyzed, although both preparations contained BMP-2, -4, and -7, the laboratory-prepared DFDBA had greater concentrations of BMP-2, the primary osteoinductive protein of the BMP family. This study suggests that even though commercially prepared DFDBA may retain proteins that have the capacity to influence cell differentiation and possibly regeneration *in vivo*, many of these proteins are lost during tissue processing.

Schwartz and colleagues[82] subsequently examined 14 batches of commercially available DFDBA from 6 bone banks. The investigators found discrepancies between and even within lots from the same bone banks in terms of particle size, surface morphometry, and pH properties. When implanted into muscle pouches of athymic mice, three of the bone bank samples formed new bone after 1 to 2

months, whereas no bone was formed after the implantation of graft materials from the other bone banks. When different preparation lots from each bone bank were analyzed, there were variations in the rate and the amount of new bone formed. A subsequent study examined 27 lots from the same bone bank, which previously had been shown to manufacture DFDBA that was consistently osteoinductive.[83] Five lots had little or no osteoinductive properties; 12 (40%) were found to be associated with new bone present in 40% or more of the surface areas examined; and only 5 lots (18.5%) produced new bone in more than 50% of the surface areas. This study suggests there is a wide variation in osteoinductive properties of DFDBA from commercial bone banks, and even among lots from the same bone bank.

Because DFDBAs have varying levels of osteoinductive properties, are there technical procedures that can maximize the osteogenic potential? Histologic sections from the controlled study by Reynolds and Bowers[84] were reviewed to study the fate of DFDBA. Approximately 72% of the grafted sites exhibited residual DFDBA particles. When comparing sites containing residual DFDBA versus those without residual DFDBA, greater amounts of new attachment formation (1.72 vs. 0.2 mm), new bone (2.33 vs. 0.23 mm), and cementum (1.74 vs. 0.23 mm) were associated with sites containing some residual DFDBA. Graft containment may thus be an important factor in influencing the regenerative response.

Can the osteoinductive potential of demineralized freeze-dried bone allograft preparation be improved? Findings suggest that preparations of DFDBA differ in their ability to induce new bone formation, and some batches may not induce any activity at all. This has resulted in studies that have attempted to describe methods for monitoring the osteogenic potential of various DFDBA batches, as well as factors that will influence the osteogenic potential of each batch.

Although the implantation of various batches of DFDBA into athymic mice may be an effective way of predicting their osteogenic potential, this system is costly and impractical. Previously, human PDL cells and ROS osteosarcoma cells have been used to predict the osteogenic potential of various batches of DFDBA.[85] The ability to induce new bone formation *in vivo* was highly correlated with cell proliferation and alkaline phosphatase production in these cells. This approach was used by Zhang and coworkers,[86] in which alkaline phosphatase activity *in vitro* was shown to be correlated with calcium uptake into the DFDBA-implanted area *in vivo*. These *in vitro* assays, together with the implantation of DFDBA into athymic mice, have indicated the influence of various factors on the osteogenic potential of a DFDBA preparation. Several tissue banks are providing *in vitro* assay and athymic mice implantation data to demonstrate the osteogenic potential of their products. Although these data are interesting, the more valuable information would be the specific data for each batch distributed. This not only would serve as quality assurance standards, but would eventually validate the usefulness of these assays.

The osteogenic potential of DFDBA appears to be dependent on the extent of demineralization. FDBA, the mineralized precursor to DFDBA, when prepared from various animal sources has been shown to be ineffective in osteoinduction. This was confirmed in a study using human DFDBA, which was effective only after demineralization. Maximum osteoinduction was observed when there was only a 2% or less residual calcium level in the DFDBA material.[87]

The osteogenic potential of DFDBA may also be dependent on the age of the bone donor. Previously, animal experiments have indicated that the osteogenic potential of rat DFDBA is age dependent. Bone harvested from middle aged donor animals had better bone forming potential than bone from younger animals, and bone formation was better in younger recipients than in older ones.[88] This finding was repeated in a study using 27 lots of human DFDBA from the same bone bank in which the age and sex of the donor for each lot was identified and each sample was implanted into athymic mice.[83] The osteogenic potential was not dependent on the sex of the bone donor, but was improved in younger compared with older donors. A study of donor age and sex suggested that DFDBA processed from donor bone of women aged 31 to 40 years and men aged 41 to 50 years possess the greatest osteoinductivity.[87] Thus, the ability to induce new bone formation appears to be age dependent, with DFDBA from older donors having the lowest osteoinductive potential. Osteoinductive potential appears not to be influenced significantly by the sex of the donor.

What is the long-term outcome of demineralized freeze-dried bone allograft-treated sites? Although there are case reports that indicate gains in clinical attachment may be maintained for up to 5 years after implantation of DFDBA in combination with expanded polytetrafluoroethylene (ePTFE) membranes, there have been few assessments of the long-term stability of sites grafted with DFDBA alone.[89] A randomized controlled study compared DFDBA-grafted and debrided sites in eight patients.[90] After 6 months, the mean bone gains for the DFDBA-grafted and the debrided sites were 2.2 and 1.1 mm, respectively. After 3 years, with 3- to 6-month intervals of periodontal maintenance therapy, the mean bone gain changed by only 0.1 mm. These data indicate that alveolar bone gain after DFDBA implantation may be maintained over 3 years.

Xenografts—anorganic bovine bone

Anorganic bovine bone (ABB) is bovine bone that has been chemically treated to remove its organic components, leaving a trabecular and porous architecture similar to human bone. It has been proposed that this bone has no osteoinductive properties, but acts as a scaffold for new bone formation (osteoconduction). Studies in rabbits and dogs have shown it to be effective in correcting experimental bone and intrabony defects.[91,92]

Animal studies provide new insights regarding the healing pattern of ABB. In a comparison of anorganic bone with bioactive glass ceramics (BGC) in a

criticalsized defect in rabbits, the anorganic bone-grafted sites were more radiopaque and had more new bone.[93] Whereas five of six anorganic bone grafted sites healed with bony union and restoration of the anatomic contour after 8 weeks, only one out of six BGC sites demonstrated similar findings. In dogs, the regenerative potential of anorganic bone plus collagen in experimental periodontal defects was evaluated at 6, 18, and 36 weeks by contact microradiography and scanning electron microscopy.[94] The anorganic bone plus collagen showed increased bone formation as compared with the flap curettage-treated sites. These two animal studies suggest that anorganic bone may be superior to BGC in experimental nonperiodontal bony defects and that, when augmented with collagen, it may be useful in correcting periodontal defects.

One study compared ABB with DFDBA in intrabony defects.[95] Significant improvement in pocket depth and clinical attachment level were observed for both graft materials after 6 months. A comparison of ABB with DFDBA indicated comparable pocket depth reduction (3.0 vs. 2.0 mm), clinical attachment level gain (3.5 vs. 2.6 mm), and bone fill (55.8% vs. 46.8% bone fill). Thus, there was no difference between the clinical healing responses with the two graft materials.

The use of ABB alone and in conjunction with GTR has been compared histologically.[95] In four anterior defects, two were grafted with ABB and two with ABB in conjunction with a bovine collagen GTR membrane. Clinical and histologic examination revealed that for the ABB^{-} and ABB^{+} GTR-treated sites, the lengths of newly formed cementum were 5.1 to 5.2 mm and 7.0 to 7.6 mm, respectively; the height of new bone was 4.2 to 4.8 mm and 4.5 to 5.3 mm, respectively. Histologically, ABB was incorporated into the new bone, which suggested that healing was osteoconductive. When used in conjunction with a GTR membrane, the new connective tissue attachment extended to the coronal level of the original intrabony defect and an increase in new bone was observed. Thus, ABB may result in gains in clinical attachment that may be accompanied by regeneration when combined with use of an occlusive membrane.

Inert biologic fillers (alloplastic materials)

Alloplastic bone grafts used in periodontics consist of ceramics, such as hydroxyapatite (HA) and TCP, and biocompatible composite polymers. These inert biological fillers represent the first generation of alloplastic bone graft materials. They have been extensively studied and were comprehensively reviewed, showing these materials to be safe and well tolerated. Although effective in procedures such as ridge preservation and ridge augmentation, these materials have been shown to be of more limited effectiveness in treating osseous defects around teeth.

Ceramics.

Ceramics consist primarily of HA[$Ca_{10}(PO_4)_6(OH)_2$] and β TCP [$Ca_{13}(PO_4)_2$]. HA is a solid calcium phosphate compound that is sintered. The physical and chemical properties of HA affect the rate of resorption and subsequently influence its clinical application. The density (dense or porous) determines the compressive strength of the graft material and the extent of vascular ingrowth. These two characteristics will influence the rate of resorption. Larger crystalline particles are nonresorbable, whereas smaller or amorphous particles are resorbed more rapidly. In general, the larger crystalline HA particles are used for ridge preservation and augmentation, and the small particles are used for periodontal applications. In clinical applications, dense HA has been shown to compare favorably with debridement in reducing probing depth (1.3 to 2.8 mm) and increasing clinical attachment gain.[96–99] A 5-year follow-up study indicated that HA-treated sites, particularly those exhibiting deep pockets (≥6 mm), were stable and less susceptible to subsequent attachment loss when compared with debrided sites.[100–102] Human histologic studies indicate that dense HA does not induce new attachment or bone formation, that pocket reduction is primarily through fibrous encapsulation of the HA particles in the intraosseous defect, and that pocket closure is through long junctional epithelium and connective tissue adhesion.[102,103] PHA has been shown to be effective in reducing probing depth and increasing attachment gain in both intraosseous defects[104–106] and Class II furcation defects.[107,108] Comparison of PHA with other grafting materials has shown PHA to produce similar clinical results to FDBA,[66] DFDBA,[76] and natural coral.[106] Other comparisons indicate that PHA is superior to DFDBA[77] and inferior to dense HA.[105] Three histologic analyses of clinical PHA-grafted defects indicated no new attachment, the presence of long junctional epithelium, and varying extents of bone associated with the PHA particles.[109–111]

β TCP is a calcium phosphate that is mixed with naphthalene at high temperatures. As the composite cools, the naphthalene evaporates, forming a porous calcium phosphate structure. Like HA, the rate of resorption is dependent on the porosity and particle size. Limited research of TCP as a periodontal graft material consists of six small noncontrolled studies with varying positive results of 1.2 to 2.8 mm bone gain and 2.3 to 2.7 mm of clinical attachment level gain.[112–115] Although animal studies indicate TCP is rapidly resorbed and replaced by bone,[116] a histologic study of human periodontal defects indicated that TCP particles are encapsulated by fibrous connective tissue and pocket closure is primarily through long junctional epithelium.[117] Thus, ceramic fillers (HA and TCP) are unlikely to result in true regeneration.

Biocompatible composite polymer.

Biocompatible composite polymer (Bioplant HTR [Bioplant Inc.; South Norwalk, CT], or "hard tissue replacement" material) consists of poly-methylmethacrylate-poly-hydroxyl-ethylmethacrylate beads coated by calcium hydroxide. This calcium hydroxide surface forms a calcium carbonate apatite when introduced into the body. HTR has been shown to be superior to

debridement alone in correcting intraosseous defects (60.8% vs. 32.2% mean defect fill)[118,119] and Class II furcation defects.[120] Clinical comparison studies have shown HTR to be equally as effective as autogenous bone grafts. Histologically, HTR rarely promotes new attachment.[121,122]

One report found that the improved clinical attachment level after implantation of HTR into furcation defects was stable after 6 years.[123] Thirteen patients with 16 maxillary and 10 mandibular grade II furcation defects were treated with HTR. Reentry after 6 to 12 months indicated an improvement of mean horizontal attachment level of 2.2 to 4.4 mm, and mean vertical attachment gain of 1.2 mm. After 6 years, the mean attachment level was maintained, indicating that implantation of HTR may be beneficial and stable in the treatment of maxillary and mandibular grade II furcations.

Calcium carbonates.

Calcium carbonates are processed natural coral skeletons (NCSs) from *Porites* coral, which can serve as resorbable bone graft substitutes. Cell culture and animal studies have indicated the material enhances osteoblastic cell attachment and growth[124] and can be converted to bone in experimental defects.[125] It enhanced healing by resorption and replacement with newly formed bone. NCS itself was not osteoinductive, but rather osteoconductive, acting as a scaffold for formation of new host bone. The first controlled study of NCS in comparison with debridement alone was performed in 20 patients with at least two defects each. In a nonpaired controlled comparison of 40 defects receiving NCS and 39 treated by debridement alone, surgical reentry after 6 months indicated a mean defect fill of 2.3 mm (67%) for the NCS-treated sites and 0.7 mm (25.9%) for those treated with debridement alone.[126] Of the sites examined, 88% of the NCS sites had more than 50% defect fill versus 18% in the control sites. This finding was confirmed by a study in which NCS was compared with PHA or debridement alone.[106] Ten patients with three intrabony defects each were treated and assessed. After 12 months, bone fill of 2.2 mm (57.4% bone fill) for NCS treatment, 2.5 mm (58.1%) for PHA treatment, and 1.1 mm (22.2%) for control were observed. These studies suggest that NCS augmentation is clinically superior to debridement alone and comparable to PHA.

Bioactive glass ceramics.

BGC are made of CaO, Na_2O, SIO_2, and P_2O_5 in the same proportions as in bone and teeth and are referred to as *45S5 bioactive glass*. This material was initially introduced as an amorphous material (Bioglass; NovaBone Products, Alachua, FL) and has been demonstrated in animal studies to regenerate bone and soft tissue attachment to teeth.[127] The material has subsequently been produced in a particulate form with a 90- to 710-µm diameter (PerioGlas; NovaBone Products, Alachua, FL) and with a 300- to 350-µm (BioGran; 3i,

Palm Beach Gardens, FL) diameter. Bioactive glass enhances bone formation by ionic dissolution of the ceramic particles such that a silica gel layer forms over the particles on contact with body fluid. Over this silica gel layer, a calcium phosphate layer forms, which is quickly converted into a hydroxycarbonate apatite layer.[128] This apatite layer has been shown to be identical to bone mineral and to provide the surface for osteoblast cell attachment and bone deposition.[129,130] The continuous ionic exchange results in dissolution of the ceramic particles such that after 1 to 3 years, the particles have been shown to be replaced by bone.[131] Only recently has clinical information been available regarding its use in the correction of periodontal defects.

In a report of a case series where BGC were placed in 17 intrabony osseous defects in 12 patients, the healing was monitored over 6 months.[132] At the end of the study, the mean probing depth was reduced 3.40 mm, and a mean attachment gain of 1.56 mm and a mean radiographic bone fill of 2.60 mm were achieved. These clinical results remained stable over a 24-month period.

In a controlled study comparing the use of BGC to debridement alone, there were significant increases in radiographic density and volume of bone in defects treated with BGC when compared with those treated only with surgical debridement.[133] Probing depth and attachment levels for both groups improved. Comparison between the groups in these parameters indicates that even though there was a greater trend toward improvement with the BGC-treated group, it was not statistically significant.

In another large, controlled, split-mouth design study, BGC was found to be superior to surgical debridement alone, as evidenced by mean probing depth reduction (4.26 vs. 3.44 mm), increased clinical attachment level (2.96 vs. 1.54 mm), and less gingival recession (1.29 vs. 1.87 mm) at 12 months.[134] Surgical reentry indicated greater defect fill with BGC (4.36 vs. 3.15 mm). This study suggests that BGC results in significant improvement in clinical parameters compared with open debridement.

BGC was compared with DFDBA in a paired study of 15 patients.[135] After 6 months, sites treated with BGC were similar to those receiving DFDBA in mean probing depth reduction (3.07 vs. 2.60 mm), mean attachment level gain (2.27 vs. 1.93 mm), and mean bone fill (2.73 vs. 2.80 mm). Surgical reentry indicated BGC resulted in 61.8% bone fill and 73.3% defect resolution, whereas DFDBA achieved 62.5% bone fill and 80.9% defect resolution. No statistical differences in soft and hard tissue improvement were observed between BGC and DFDBA during the 6-month study.

Guided Tissue Regeneration/Guided Bone Regeneration

Our current understanding of periodontal healing is based on a hypothesis by Melcher,[2] who proposed that the cell type that repopulates the exposed root surface at the periodontal repair site will define the nature of the attachment or repair that takes place. If mesenchymal cells from the PDL or perivascular region of the bone proliferate and colonize the root surface, regeneration occurs. Alternatively, if lost tissue is replaced by the surrounding tissue to form a scar, repair occurs. The anatomy of the scar is dependent on the cell types that predominate the defect. The four cell types of concern in the periodontium are gingival epithelial cells, mesenchymal cells from gingival connective tissue, alveolar bone cells, and PDL cells (see Fig. 25-1). If epithelial cells proliferate along the root surface, a long junctional epithelium will result. If gingival connective tissue populates the root surface, a connective tissue attachment will form and root resorption may occur. If bone cells migrate and adhere to the root surface, root resorption and ankylosis occur. Root resorption is much more common in animal models than it is in humans.

Animal models studying GTR have confirmed the importance of PDL cells as progenitor cells for periodontal regeneration.[136,137] Evaluation of cell proliferation kinetics revealed that both the PDL and perivascular cells from the bone proliferate and migrate into the osseous defect to form the early healing tissue.[138] Melcher[139] has amended his original hypothesis to include the contribution of the perivascular cells of the bone in periodontal regeneration—that is, cells from both the PDL and alveolar bone are important in formation of new bone, cementum, and functionally oriented PDL (regeneration). This current theory influences much of our therapeutic approaches toward management of periodontal defects.

A classic nonhuman primate study evaluated histologic healing after four different treatments: a modified Widman flap procedure, a flap procedure with a frozen autologous bone graft, a flap procedure with a TCP graft, and periodontal root planing and soft tissue curettage.[17] All four therapies resulted in repair in the form of long junctional epithelium with limited regeneration restricted to the base of the periodontal defects. These results suggest that the apical migration of epithelial cells occurs more rapidly than the colonization of the reparative surfaces by other cell types.

Early clinical approaches toward epithelial exclusion suggested this approach may enhance regeneration.[140] Denudation procedures were used to excise all interdental soft tissue, granulation tissue, and calculus from three-walled intrabony defects. Surgical dressings were applied to prevent epithelial ingrowth from the surrounding wound margins. Using this approach, two studies[141,142] reported a mean clinical attachment level gain of 2.44 mm and a mean defect fill of 47.5%. Defect improvement resulted from a combination of crestal resorption (mean, 0.48 mm) and defect repair (mean, 2.55 mm). These results are similar to other osseous grafting studies in terms of percent of defect fill.

A series of experiments and clinical studies[143–146] demonstrated that if the apical migration of epithelial cells can be impeded and PDL cells allowed to repopulate

the root surface, regeneration will occur. The use of an occlusive membrane barrier to promote the formation of new periodontium is called GTR (guided tissue regeneration).

As early animal studies confirmed Melcher's postulate that the cells that populate the root surface during healing will define the healing tissue, this approach was developed into clinical procedures for human defects. To promote the proliferation of PDL cells, membrane barriers were used to exclude epithelial, bone, and gingival connective tissue cells. Classically, GTR is associated with the use of membranes, either nonresorbable or resorbable (Box 25-2). However, other forms of barriers also have been used.

Nonresorbable membranes

In classic animal and human studies demonstrating the efficacy of GTR, cellulose acetate filters were used. As this technique became more prevalent, the first commercial membrane was produced from ePTFE. This membrane has all the properties necessary for GTR barriers; for example, it (1) is a cellular barrier, (2) is biocompatible, (3) provides space for the healing tissue, (4) permits tissue integration, and (5) is clinically manageable. Much of our current understanding of GTR is based on studies using ePTFE membranes. Although currently used less frequently, Eptfe membranes are still popular for guided bone regeneration (GBR) and ridge preservation; therefore, it is important to understand the clinical procedures for managing these membranes.

Box 25-2 Guided Tissue Regeneration Membranes

Nonbioresorbable membranes

1. Expanded polytetrafluoroethylene (ePTFE)
2. Miscellaneous membranes (Millipore membrane, rubber dam)

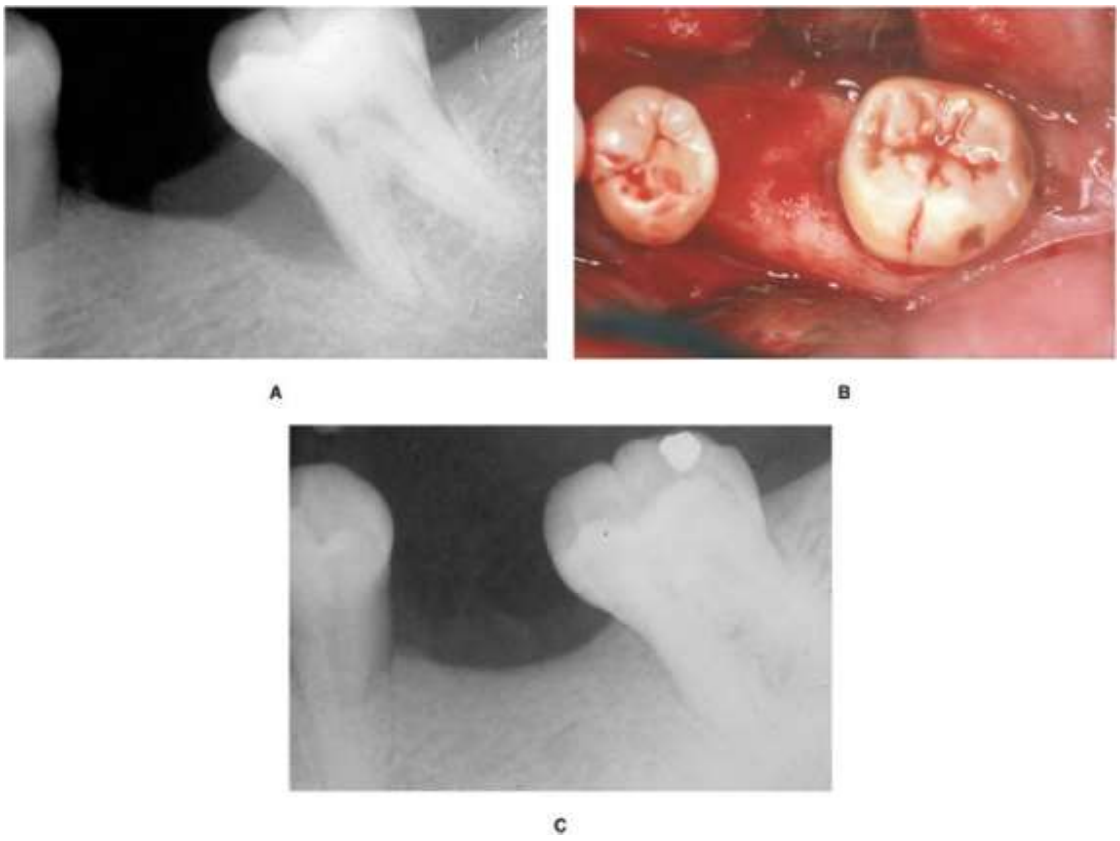
Bioresorbable membranes

1. Synthetic polymers
   - Polyurethane
   - Polylactic acid
   - Lactide/glycolide copolymers (e.g., polyglactin-910)
   - Polylactic acid blended with citric acid ester
2. Natural biomaterials (e.g., collagen)

3. Calcium sulfate

The clinical effectiveness of ePTFE membranes is dependent on technique. Preservation of the keratinized gingiva and a relatively thick overlying surgical flap are critical to avoid perforation of the flap by the membrane during healing. After flaps have been reflected in the surgical area, the defect is degranulated and the root surface scaled and root planed. The ePTFE membrane is trimmed to adapt to tooth configuration, secured by ePTFE sutures, and the flap is repositioned. Notably, although much of the emphasis in the literature is on adapting the membrane to the defect, no membrane can ever be perfectly adapted. Despite the presence of gaps between the membrane and the root surface, these membranes seem to work. After membrane placement, healing is allowed to proceed for 4 to 6 weeks. Barring any membrane exposure, a second surgery is performed to remove the membrane. During this removal, the healing tissue often appears reddish and granulomatous, although more mature bonelike tissue is sometimes noted. After membrane removal, the area should not be probed for 3 to 6 months. Radiographic evidence of bone fill is usually present after 6 months and should continue during the course of 1 year (Fig. 25-4).

Figure 25-4.



Radiographs and clinical photograph of a guided tissue regeneration case using a nonresorbable expanded polytetrafluoroethylene (ePTFE) membrane.

The mesially inclined molar is associated with a three-walled intraosseous defect (**A**). The defect was filled with demineralized freeze-dried bone allograft, and ePTFE membrane was used (**B**). Membrane became exposed after 8 weeks and was removed 2 weeks later. Radiographic "fill" was approximately 50% after 6 months, and maximum fill was present after 12 months (**C**).

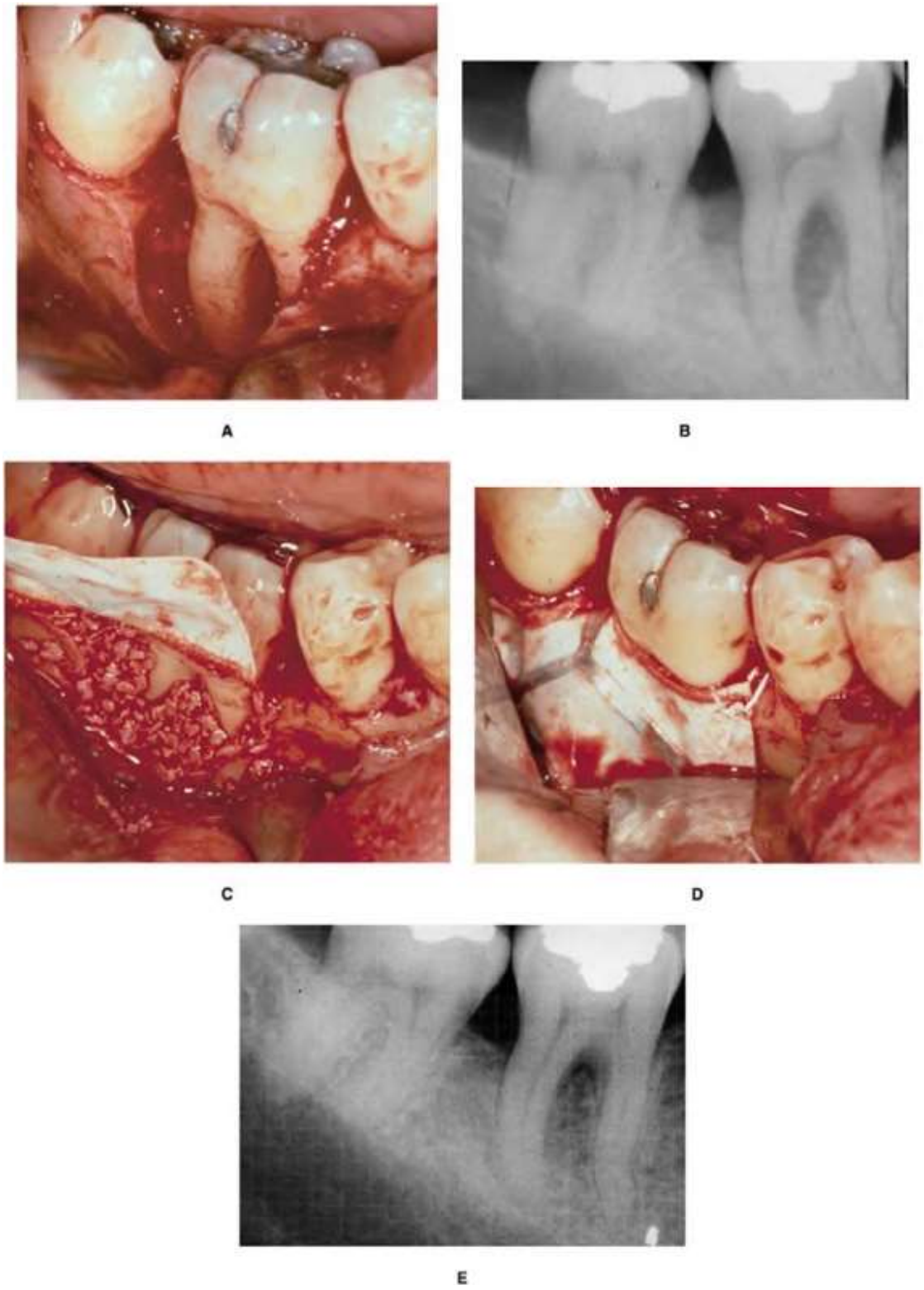
Clinical studies have shown that ePTFE membranes used in GTR procedures are more effective than surgical debridement alone in correcting intrabony defects.[147–154] In intrabony and furcation defects, there are gains in clinical attachment level (3 to 6 mm), improved bone levels (2.4 to 4.8 mm), and probing depth reductions (3.5 to 6 mm). Studies have demonstrated that these regenerative results can be maintained during the course of several years.[89,155–157]

The advent of titanium-reinforced ePTFE membranes allowed for the formation of larger spaces, thus permitting correction of larger defects (Fig. 25-5).[158] These membranes are embedded with strips of titanium that can be bent and shaped to fit the bony defects and prevent collapse of the membrane into the defect. This resulted in significant clinical improvements using titanium-reinforced ePTFE compared with ePTFE.

To determine how regeneration can be enhanced with GTR technique, the prolonged retention of ePTFE membranes was evaluated.[159] After allowing the membrane to be retained for 4 months, surgical reentry after 1 year determined that the mean bone fill of intrabony defects was 95%. This suggests that prolonged retention of a barrier membrane is desirable if no tissue perforation is present. This is consistent with many clinical reports of the improved bone quality associated with GBR in implant site development.

The major problem with using nonresorbable membranes is that the membrane may become exposed to the oral environment during healing. On exposure, the membrane is contaminated and colonized by oral microflora.[160–162] Several studies have shown that contamination of the surgical field can result in decreased formation of new attachment.[16,163,164] If the membrane is exposed, the infection can be temporarily managed with topical application of chlorhexidine. This may minimize the infection and extend the time the membrane can be retained in place. However, any sign of frank infection such as swelling or pus formation suggests the membrane should be removed.

Figure 25-5.



Clinical photographs and radiographs of a guided tissue regeneration case using titanium-reinforced expanded polytetrafluoroethylene membrane. The

osseous defect was along the distal interproximal area wrapping buccally over the furcation (**A** and **B**). Tooth #30 was vital. To prevent the membrane from collapsing into the osseus defect and over the root surfaces, demineralized freeze-dried bone allograft was placed in the defect and the titanium-reinforced membrane was molded to provide a larger space for regeneration (**C** and **D**). One year later, the radiographic and clinical signs are consistent with achieving significant bone fill (**E**).

Bioresorbable membranes

For many clinicians, bioresorbable membranes have replaced the routine use of ePTFE membranes in GTR. There are basically three types of bioresorbable membranes: (1) polyglycoside synthetic polymers (i.e., polylactic acid, polylactate/polygalactide copolymers), (2) collagen, and (3) calcium sulfate. Polyglycoside membranes degrade as the result of random nonenzymatic cleavage of the polymer, producing polylactide and polyglycolide, which are converted to lactic acid and pyruvate, respectively, and metabolized by the enzymes of the Krebs cycle. Collagen membranes currently available are of porcine or bovine origin, and consist of either type I collagen or a combination of type I and type III collagen. Collagen membranes are degraded by collagenases and subsequently by gelatinases and peptidase. There has been a resurgence in the use of calcium sulfate as a regeneration material because it can be used as a pavable resorbable barrier when used in combination with bone or bone substitutes. The calcium sulfate is bioresorbed through a giant cell reaction. Several features make these bioresorbable membranes easier to manage clinically: (1) they are more tissue compatible than nonresorbable membranes; (2) the timing for resorption can be regulated by the amount of cross-linkage in the synthetic polymer and collagen membrane or the amount of heat-processed calcium sulfate chips in calcium sulfate barrier; and (3) a second surgical procedure is not required to retrieve the nonresorbable membrane. A disadvantage of many resorbable membranes is a relative lack of rigidity, because resorbable membranes, unlike titanium-reinforced ePTFE membranes, have no embedded support structures.

In a 1-year GTR study comparing the use of bioresorbable membranes (polylactate/polygalactate copolymer), ePTFE membranes, or surgical debridement alone, significant gains in clinical attachment level were observed in all three groups.[165] There was no difference in clinical attachment level gain between the two membrane groups, with both of them gaining 2 mm or more. In both membrane groups, 83% of the sites improved 4 mm or more, which was significantly better than the surgical debridement control group. These findings indicate GTR procedures are equally effective using resorbable and nonresorbable membranes. This finding has been confirmed by other investigators.[166–168]

A large multicenter clinical study reported the use of bioresorbable membranes in 203 consecutively treated intrabony defects.[169] After 1 year, investigators found that clinical attachment level improved by 79%, and 78% of the sites improved by

4 mm or more. An average of 3 mm of bone fill was measured radiographically. Compromised clinical results occurred in cases where membranes became exposed to the oral environment or where patients had poor plaque control.

Use of guided tissue regeneration with bone grafting

Although regeneration may be attempted with various graft materials used alone or with membranes alone, combinations of the two may also be indicated. The use of GTR in conjunction with various regenerative approaches has been attempted with reported success. In a large case series using GTR in combination with root conditioning and DFDBA, significant gains in clinical attachment level were observed in a variety of furcation and intrabony defects.[153] Importantly, the regenerated results were stable over 5 years.[89] Others have reported similar positive clinical results with DFDBA alone.[170] When this combination was used and studied histologically, the amount of newly regenerated attachment varied from 0 to 1.7 mm.[171] In a splitmouth paired control study comparing GTR versus GTR with DFDBA, both groups had improved bone fill, but there were no statistically significant differences between the two groups.[172,173] A similar study was performed comparing GTR alone with GTR plus HA-collagen grafts.[154] Improved results were seen in both groups, with no significant differences between groups. These and other studies suggest that GTR techniques may be somewhat improved with the use of bone grafts or other defect fillers. In a comprehensive, systematic review of human data, use of a bone augmentation material in combination with a membrane was shown to provide clinically superior results compared to use of a membrane alone in the treatment of molar furcation defects.[174] In treating interproximal intrabony defects, the two approaches gave similar results.

The use of GTR with bone grafting has been applied with the use of calcium sulfate. Calcium sulfate has been safely used in periodontics for the last four decades.[175–177] Animal studies indicated that calcium sulfate can create a "sealing" effect that permits orderly bone replacement of the osseous defect. The calcium sulfate resorption time averaged 2 to 4 weeks.[175] Early clinical application to periodontal defects reported favorable results, but it did not demonstrate any capacity for osteoinduction.[178,179] Because the barrier effect was minimal, this technique was abandoned until its revival this past decade. Sottosanti[180] altered the technique to gain adequate time for regeneration by modifying the use of calcium sulfate to include a bone graft. The technique involves two basic components. The first component is composite graft of approximately 80% DFDBA and 20% calcium sulfate, which is placed into the defect. Over this composite graft is a second placement of a calcium sulfate barrier. The advantage of this technique is that the material is highly tissue compatible, it permits the management of large irregularly shaped defects, and gaps in flap coverage do not appear to be significant. Several clinical case reports and series have suggested

this as a viable technique,[181–183] but no large clinical, controlled, or comparable studies are available.

Using guided tissue regeneration principles for implant site development (guided bone regeneration)

The principle of selective cell repopulation has been useful in enhancing site development for implant placement. Whereas GTR requires the regeneration of bone, PDL, and cementum to form a new periodontal apparatus, the requirements for implant site development are less complicated in that only bone formation needs to be enhanced. By using a barrier membrane at an extraction site or a deficient alveolar ridge, bone can be regenerated. At the time of tooth extraction, the socket can be augmented with a graft material and "sealed" with a barrier membrane. In some cases, a membrane may be used without graft material in the socket. This procedure is termed ridge preservation (see also Chapters 21 and 26). Similarly, an alveolar ridge with a volumetric deficiency can be improved with the use of graft material and a barrier. This procedure is termed GBR (guided bone regeneration) and is a commonly used technique for osseous ridge augmentation. Both of these approaches use the barrier concept to selectively permit osteoprogenitor cells to colonize the site such that an increased volume of bone may be formed.

In ridge preservation, the need for a barrier membrane is highly dependent on the nature of the alveolar housing. In a site with thick gingiva and a thick labial alveolar plate, there is minimal postextraction remodeling and the management required is minimal. In these cases, ridge preservation may not be needed after extraction. Alternatively, the thin gingiva case with a thin labial plate is susceptible to remodeling. As the ridge heals, there is a tendency for the ridge to remodel apically and lingually, resulting in a vertical and a horizontal deficiency. To prevent this, ridge preservation procedures can minimize ridge atrophy, especially in the vertical dimension. This is especially important because most ridge augmentation techniques work fairly predictably in correcting horizontal defects, but they are more limited in restoring the vertical dimension. As a preparatory procedure, ridge preservation can minimize the number of subsequent augmentation procedures needed. With this technique, it is critical to extract the tooth atraumatically. The socket is degranulated thoroughly and grafted. Though various graft materials have been advocated, it is important to remember that an implant needs to be placed in this space approximately 3 to 6 months after extraction. The ideal graft material needs to act as a scaffold for new bone formation, and also to be minimal in volume at the time of implant placement. This is important to maximize the amount of bone available for osseointegration. It has been noted that when implants are placed in grafted sites, non-resorbed graft materials are displaced laterally and do not interact with the implant surface. If the newly healed site is predominately filled with residual graft material, the

site may not be structurally ideal for osseointegration and site integrity. Consequently, some have advocated that no graft material be placed and only a membrane barrier should be used. In these cases, it is hoped that the socket will fill completely with new host bone.

Several types of membranes, as well as calcium sulfate barriers, have been reported to be effective in "sealing" the socket. When used, ridge preservation minimizes the amount of remodeling. Invariably, there will be some degree of ridge resorption, and the patient should be advised that further treatment such as ridge augmentation may be needed to develop the ideal implant placement site (see Chapter 26).

GBR is one of the many approaches for ridge augmentation. In this technique, the deficient alveolar site is surgically exposed and all soft tissue adherent to bone is removed. Many clinicians have advocated perforating the cortical plates to open the marrow spaces and allow for osteoprogenitor cell migration into the site. Graft materials are used to serve as volumetric scaffolds and a membrane is used to "seal" the area. Membrane requirements that appear important include its ability to be maintained during the course of treatment and its ability to support the increased tissue dimension. Early studies focused on the use of ePTFE because many of the resorbable membranes initially on the market were not intact after a few months. The advent of titanium-reinforced ePTFE membranes also helped with maintaining the space under the membrane required for regeneration. The difficulty with ePTFE membranes is that their stiffness and thickness often resulted in soft tissue perforation. The ensuing infection often compromised the amount of regeneration achieved. More recently, the more tissue-compatible resorbable membranes have been modified to slow their resorption, so the barrier effect can be maintained up to 6 months. Regardless of the type of membrane used, the difficulty with this approach of ridge augmentation is that it is not highly predictable, the volume of bone regeneration attainable is limited, and the ridge can be improved mainly in the horizontal dimension. In situations where extensive augmentation is needed (≥3 mm), other augmentation techniques such as alveolar monocortical grafts or distraction osteogenesis should be considered.

Ridge preservation and GBR are best used at the time of extraction to preserve and possibly improve the alveolar ridge in preparation for implant placement (Fig. 25-6). Importantly, after these procedures, additional augmentative procedures may be necessary. These options and other implant site development approaches are discussed in detail in Chapter 26.

NEW APPROACHES TO PERIODONTAL REGENERATION

Experimental and clinical studies on GTR have validated Melcher's postulate that the germinal cell type that colonizes the periodontal wound healing site will determine the fate of the healing tissue. Although the use of a barrier membrane enhances our ability to regenerate the periodontium, its efficacy is limited to certain periodontal

defects. Periodontal regeneration is unpredictable in circumferential, one- or two-wall intraosseous defects, and in Class III and advanced Class II furcation defects. This past decade, research has focused on two main approaches involving the use of biological mediators to selectively enhance cellular repopulation of the periodontal wound. The first approach involves the use of peptide sequences, protein preparations, and growth factors to regenerate tissues through the principle of biomimicry. Biomimetics is the science of constructing or mimicking natural processes or tissues, with the expectation that the regeneration cascade will proceed spontaneously. Enamel matrix derivative (EMD), platelet-rich plasma (PRP) preparation-fibrin glue, and growth factors such as platelet-derived growth factor (PDGF) purportedly function in this fashion. The second approach involves the use of growth differentiation factors to enhance periodontal regeneration. BMPs are differentiation factors that have been studied extensively for periodontal and bone regeneration. Several of these growth factors and derivatives are present in bone and teeth (Table 25-2), and they have been shown to have *in vitro* effects on various types of cells within the periodontium (Table 25-3).

Enamel Matrix Derivative

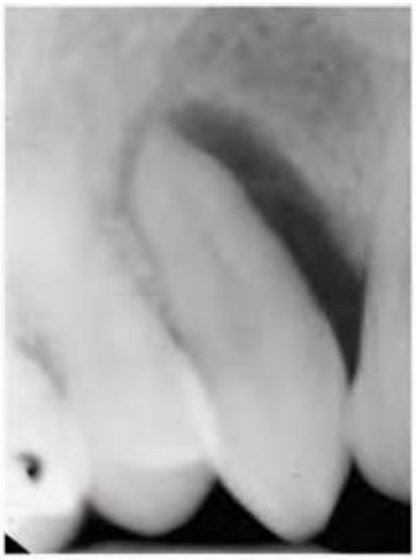
EMD harvested from developing porcine teeth has been reported to induce periodontal regeneration. The rationale for the mechanism of action is that EMD contains a protein preparation that mimics the matrix proteins that induce cementogenesis. During root development, the Hertwig's epithelial sheath deposits enamel matrix proteins on the newly formed root dentin surface. These proteins stimulate the differentiation of surrounding mesenchymal cells into cementoblasts, which form acellular cementum.[184] Once a new cementum layer is formed, collagen fibers form in the adjacent PDL, attaching into the new cementum.[185,186]

EMD is an acetic acid extracted protein preparation from developing porcine tooth buds that contains a mixture of low molecular weight proteins. The major constituents are amelogenins, which are highly hydrophobic proteins that aggregate and serve as a nidus for crystallization. Other proteins identified include ameloblastin and enamelin. This protein preparation uses propylene glycol alginate (PGA) as a carrier. The EMD-containing PGA remains highly viscous when stored in the cold or at room temperature. Once it is applied to the tissue at a neutral pH and at body temperature, the PGA carrier decreases in viscosity, and the EMD preparation precipitates. EMD is absorbed into the HA and collagen fibers of the root surface, where it induces cementum formation followed by periodontal regeneration.

In vitro studies indicate EMD may influence the cellular activities of the various cell types in the periodontium. When PDL cells are exposed to EMD, the cells exhibit enhanced protein production, cell proliferation, and the ability to promote mineral nodule formation.[187] More recently, cementoblasts treated with EMD and osteoblasts in cell culture increased cell proliferation, altered the gene expression of osteocalcin and osteopontin, and inhibited mineral nodule formation.[188]

Understanding how cells respond to EMD may elucidate how biomimetic agents work in general. It is only through this understanding that there can be a more predictable clinical therapy.

Figure 25-6.



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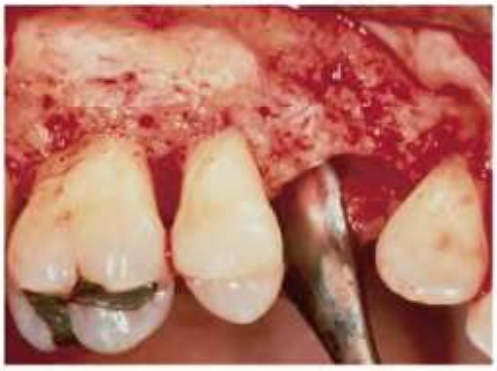

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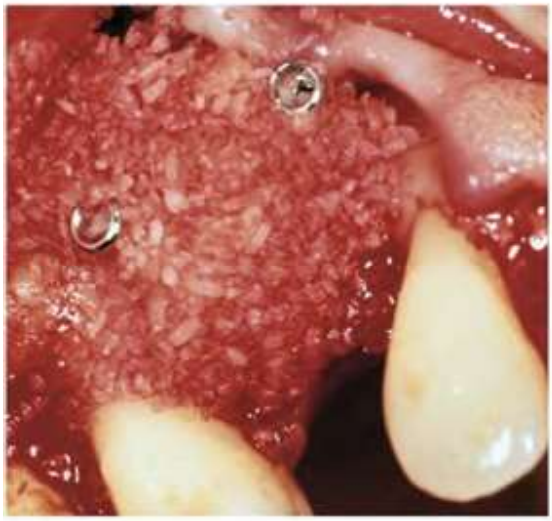

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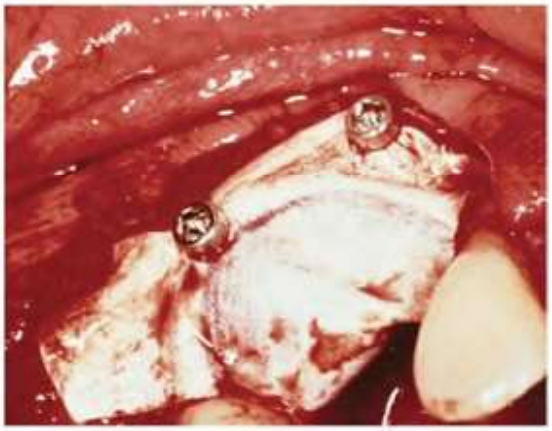

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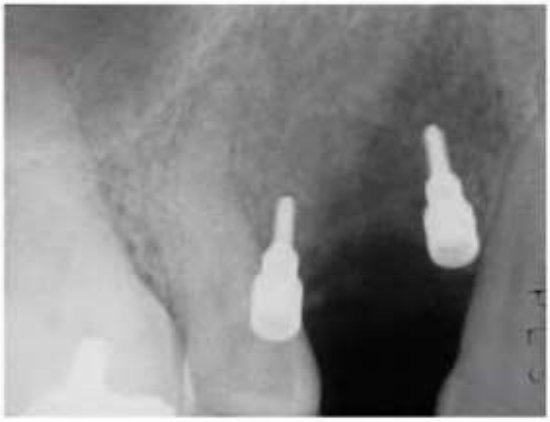

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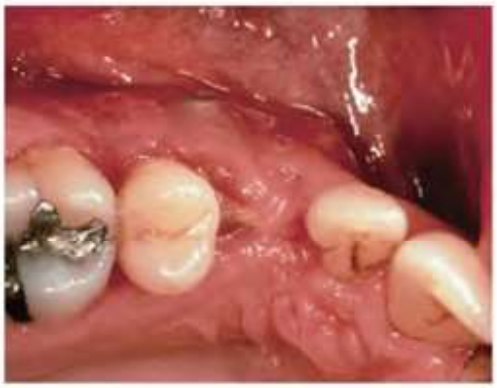

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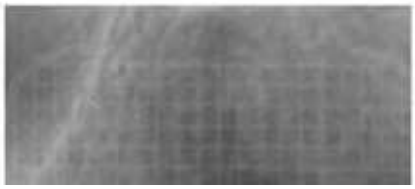


Tooth #6 has a history of labial draining fistula and was deemed hopeless (**A**). The treatment plan was to extract the tooth and prepare the site for a dental implant by using a combination of socket wall preservation and guided bone regeneration. The tooth was extracted and the socket degranulated (**B**), and the labial defect was managed with tenting pins and demineralized freeze-dried bone allograft (DFDBA) (**C**). The tenting pins and DFDBA provide space for new bone formation. An expanded polytetrafluoroethylene membrane was placed over the augmented area (**D**). This functionally preserved the socket walls and augmented the labial aspect to provide more bone on the labial aspect for implant placement. After 3 months of healing, radiographic evidence of fill was present (**E**). The two tenting pins are clearly visible on the radiograph. These pins will be removed at the next surgery. At 6 months after extraction, the ridge is clinically healed (**F**) and radiographic evidence of increased mineralization was present (**G**). On reentry for implant placement surgery, the site was successfully augmented to allow the implant to be placed in an ideal position (**H**).

In an animal study, experimental dehiscence defects were created with bilateral removal of the alveolar bone, PDL, and cementum.[189] Before repositioning the flap, one side was treated with acid-etching and EMD, whereas the control side was acid-etched only. After 8 weeks, histologic examination of the specimens indicated that the EMD-treated side generally showed no gingival recession or formation of a long junctional epithelium, and 60% to 70% of the surface was covered with regenerated acellular cementum. The control sites displayed gingival recession with only 10% of surfaces regenerated.

The histologic finding of EMD-induced periodontal regeneration has been confirmed in a clinical case report.[190] A mandibular lateral incisor destined for orthodontic extraction was treated with acid-etching and EMD. After 4 months, the tooth was extracted and examined histologically. Regenerated cementum covered 73% of the defect and regenerated alveolar bone covered 65%. This histologic finding has been confirmed in another case series.[191,192]

TABLE 25-2 Growth Factors in Bone Matrix

BMP, *bone morphogenetic protein*; IGF, *insulin-like growth factor*; PDGF, *platelet-derived growth factor*; TGF, *transforming growth factor*.

Growth Factors in Bone Matrix	Size, kD	Bone Content, mg/kg	Source in Bones (osteoblast/serum)
TGF-β	35	200	+/+
BMP-1 to -12	16-30	2-5	+/−
PDGF AA, AB, BB	36	50	+/+
IGF-1, -2	7, 6	400	+/+

TABLE 25-3 *In Vitro* Effects of Growth Factors on Periodontal Ligament Cells and Osteoblasts

(−), *inhibitory effect*; *0*, *no effect*; (+), *slight effect*; (++), *moderate effect*; (+++), *strong effect*; BMP, *bone morphogenetic protein*; IGF, *insulin-like growth factor*; ND, *not done*; PDGF, *platelet-derived growth factor*; PDL, *periodontal ligament*; TGF, *transforming growth factor*.

	TGF-β	BMP-2, -3	BMP-7	PDGF	IGF-1,-2
PDL cells					
• Cell proliferation	−	++	++	++	+
• Chemotaxis	0	+	+	++	++
• Collagen synthesis	+	+	+	+	+
• Protein synthesis	+	+	+	+	+
Osteoblasts					
• Cell proliferation	+++	0	+	++	++
• Chemotaxis	+++	+	ND	+++	+
• Collagen synthesis	++	0	+	0	+
• Protein synthesis	+/−	ND	0	0	0
• Alkaline phosphatase synthesis	+/−	++	+++	0	0

In a multicenter study, 33 patients with at least two intrabony defects were treated in a split-mouth design in which the experimental site was acid-etched and EMD was applied to the denuded root surfaces.[193] At the control site, a placebo was applied. Patients were examined at 8, 16, and 36 months after surgery. Increased bone fill of the osseous defect was observed over time for 25 of the 27 (93%) EMD-treated teeth, but no bone fill was detected in the controls. The mean radiographic bone fill was greater for the EMD-treated defect compared with the control sites (2.7 vs. 0.7 mm). Statistically significant improvements were observed for EMD-treated sites over control sites in mean pocket reduction (3.1 vs. 2.3 mm) and mean attachment level gain (2.2 vs. 1.7 mm). These clinical findings have been supported by several other studies.[194–196]

The biosafety of EMD was tested on 107 patients who were treated with EMD at two separate visits.[197,198] No adverse clinical or immunologic reactions were observed. None of the serum samples analyzed for total and anti-EMD antibodies indicated deviations from established baseline ranges. After 3 years of clinical use, approximately half the patients were reevaluated and there was no report of adverse reaction. This study suggests that the immunogenic potential of EMD is extremely low when applied in conjunction with periodontal surgery.

There have been four studies comparing the use of EMD alone or in conjunction with other regenerative approaches. When EMD treatment was compared with GTR using bioresorbable membranes, the clinical results were comparable and stable over a 4-year period.[199] No significant difference was found when EMD with BGC was compared with bioactive glass as the sole grafting material.[200] Similar comparable results were found when EMD was used in conjunction with anorganic bone graft material.[201,202]

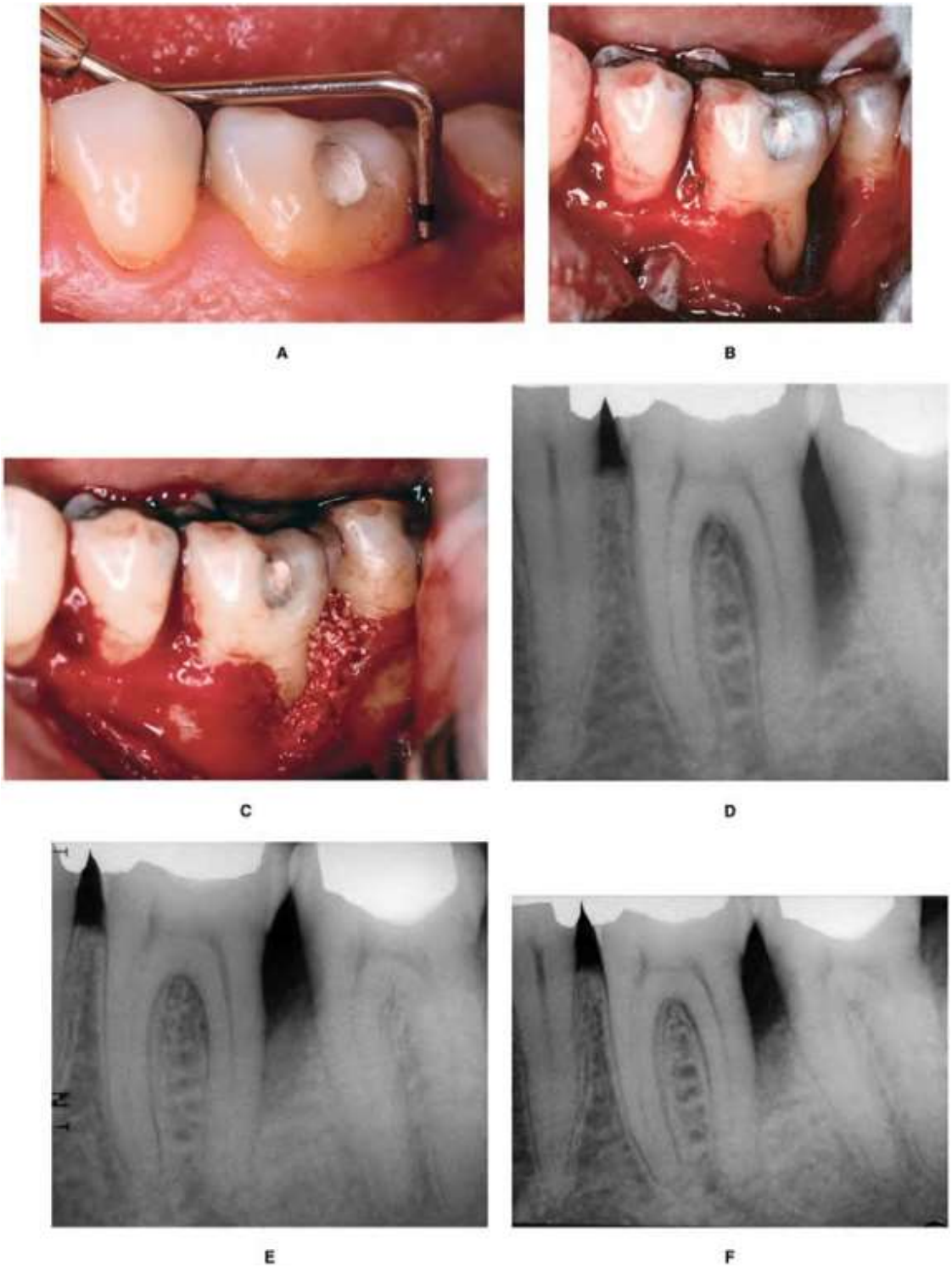
Growth Factors for Biomimicry

Growth factors are naturally occurring proteins that regulate various aspects of cell growth and development.[203,204] Several growth factors have been identified and characterized. Several of these growth factors are found in the bone matrix (Table 25-2). In wound healing, these growth factors modulate cell proliferation, migration, extracellular matrix formation, and other functions of selected cell types. In addition, some growth factors may also function as cell differentiation factors. In periodontal regeneration, much of the focus has been on PDGF, insulin-like growth factor (IGF), and, more recently, PRP preparation.

Most of the information about growth factors comes from cell culture experiments. Before biotechnology, crude preparations of growth factors were applied to various cells in culture, and their effects on selected target cell types (i.e., fibroblasts, osteoblasts, epithelial cells, and others), cell proliferation and function, extracellular matrix formation, and phenotypic expression were studied (Table 25-3).

PDGF is one of the early growth factors studied for its effect on wound healing because it is a potent mitogenic and chemotactic factor for mesenchymal cells. IGFs are growth factors that are highly homologous with proinsulin. Two of the most well characterized growth factors in this group are IGF-1 and IGF-2, which are somewhat similar, but have different receptors and properties. IGF-1 has been shown to be an effective chemotactic agent and mitogen for osteoblasts and PDL cells. Early cell culture experiments using PDGF and IGF-1 indicated that these two growth factors produced greater mitogenic responses when used together than when used individually. This synergistic effect resulted in distinguishing growth factors as either competence growth factors, which prime the cell to enter the cell proliferation cycle, or as progression growth factors, which are required for cell division. In these classic experiments, PDGF was determined to be a competence factor and IGF-1 to be a progression factor.

Figure 25-7.



A case from the clinical trial for the use of recombinant human platelet derived growth factor (rhPDGF) for the treatment of periodontal defects. Initial probing depth was 14 mm and the tooth tested vital (**A**). After flap reflection and degranulation, the osseous defect was 9-mm-deep and 4-mm-wide (**B**). The root

surface was treated with rhPDGF and the defect was filled with rhPDGF-tricalcium phosphate (**C**). Note that no guided tissue regeneration (GTR) membrane was used. The radiographs indicate increased radiopacity from the initial surgical radiograph (**D**) to the 3-month (**E**) and 6-month (**F**) postsurgical radiographs. This pattern of radiographic improvement is approximately twice as fast as those observed with GTR cases. Final probing depth was 4 mm, with 4 mm of recession. The gain in clinical attachment level was 6 mm. Histologic evidence of regeneration for a similarly treated case is presented in Figure 25-3.

Using the information from these cell biology experiments, PDGF and IGF-1 were topically applied to periodontally diseased root surfaces in beagle dogs.[205,206] Substantial amounts of new bone, cementum, and PDL were present after 2 weeks. The results of this study were subsequently confirmed in three other studies using beagles and experimentally induced periodontitis in nonhuman primates.[207–209] A human clinical trial was conducted using recombinant human PDGF/recombinant human IGF-1 (rhPDGF/rhIGF-1).[210] Using a split-mouth design, defects were treated with either a low dose (50 µg/ml) or high dose (150 µg/ml) of rhPDGF/rhIGF-1. After 9 months, high-dose rhPDGF/rhIGF-1 induced 2.08 mm of new bone with 43.2% osseous defect fill, as compared with 0.75-mm vertical bone height and 18.5% bone fill in placebo controls. Low-dose rhPDGF/rhIGF-1 was statistically similar to control.

Simultaneous with the human clinical trial, a primate study examined the regenerative effects of PDGF/IGF in combination or individually.[209] PDGF alone was found to be as effective as the PDGF/IGF combination in producing new attachment after 3 months. No significant effect was found when IGF was used alone. This study suggests that IGF may not be important at the dose level tested. A multicenter clinical trial of rhPDGF is currently being evaluated (Fig. 25-7).

The animal studies and human clinical trials suggest that PDGF may be useful in enhancing periodontal regeneration. Although encouraging, the regenerative response reported in the first clinical trial is not dissimilar to that found with GTR or with the use of bone graft materials. Additional clinical trials are needed to test greater dosages of PDGF and PDGF in combination with GTR to determine whether regeneration can be enhanced. Lastly, the role of IGF-1 needs to be elucidated, and studies need to be conducted to determine whether the effects of competence and progression growth factors are *in vitro* events only, or a clinical phenomenon as well.

Platelet-Rich Plasma Preparation

The use of PRP preparation as a source of growth factors in bone and periodontal regeneration has been proposed.[211] In this approach, autologous blood is drawn and separated into three fractions: platelet-poor plasma (fibrin glue or adhesive), PRP, and red blood cells. Platelets are enriched by 338% in the PRP preparation and concentrations of PDGF and TGF-β in PRP are 41.1 and 45.9 ng/ml,

respectively.[212] Monoclonal antibodies have identified the presence of PDGF, IGF, and TGF-β in the cytoplasmic granules of platelets. This preparation also contains a high concentration of fibrinogen. In clinical use, calcium and thrombin are added to the PRP preparation to activate the proteolytic cleavage of fibrinogen into fibrin. Fibrin formation initiates clot formation, which, in turn, initiates wound healing. Although many case reports attribute improved healing results to these growth factors, it is questionable whether the concentrations used are adequate to elicit clinically measurable results. The level of PDGF in PRP is 3000-fold less than that reported to be effective in other studies of PDGF.[210] Alternatively, the accelerated healing may be the result of the presence of a fibrin clot, which stabilizes the early wound healing matrix.

Differentiation Factors: Bone Morphogenetic Proteins

Bone morphogenetic proteins (BMP) are a group of regulatory glycoproteins that are members of the TGF-β superfamily. These molecules primarily stimulate differentiation of mesenchymal stem cells into chondroblasts and osteoblasts. At least seven BMPs have been isolated from bovine and human sources. In the field of periodontal regeneration, much of the research interest has focused on BMP-2 (OP-2), BMP-3 (osteogenin), and BMP-7 (OP1).[213]

The osteoinductive effect of BMPs was characterized by using crude protein preparations derived from decalcified bone. When these crude preparations were placed in muscle or subdermal pouches, an ectopic focal formation of cartilage was present after 12 days, and bone was present after 28 days. The induction of mesenchymal stem cell differentiation to recapitulate endochondral bone formation stimulated clinical interest in using bone preparations (FDBA and DFDBA) as osteogenic graft materials. However, when the actual concentration of BMPs in commercial bone preparations was measured, the amount present was quite low. Approximately 10 kg of bovine bone yields 2 μg of BMP.[82] This has resulted in research efforts to purify, identify, and characterize BMPs so they can be synthetically produced by recombinant DNA technology.

Experiments using crude and recombinant BMPs have provided insight as to their potential use. Crude preparations of BMP-2 and BMP-3 applied in surgically induced furcation defects appeared to stimulate periodontal regeneration.[214] Studies have used recombinant human BMPs (rhBMPs) to determine their potential for correcting horizontal bone loss and intrabony, furcation, and fenestration defects.[215–219] When rhBMP-2 was used in horizontal periodontal bony defects, the gains in bone and cementum were 3.5 and 1.6 mm, respectively, compared with 0.8 and 0.4 mm for controls.[217] Histologic analysis revealed periodontal regeneration with areas of ankylosis. Contrary to these findings, BMP-7 augmentation resulted in a significant increase in periodontal regeneration without any ankylosis.[217] Healing through ankylosis has been a concern; therefore, most of the research using rhBMPs has involved its effect in stimulating new bone formation through GBR before or in conjunction with implant placement, where ankylosis is of no concern.[220–227]

Gene Therapy for Correcting Periodontal Defects

Major limitations associated with the use of growth and differentiation factors include their short biological halflives. The factors, once applied, are subject to proteolytic breakdown and receptor binding problems and are dependent on the stability of the carrier system. Gene therapy can be used for extended local delivery of these factors. Gene delivery of PDGF was accomplished with the successful transfer of the PDGF gene into the cementoblast and other periodontal cell types.[228] This study demonstrated that gene delivery of PDGF stimulated more cementoblast activity than a single application of recombinant PDGF. In another report, periodontal wounds were transduced effectively by the use of gene transfer.[229] The use of gene delivery offers a new approach to delivering growth factors. The safety and efficacy for using gene therapy for regeneration have yet to be evaluated.

Factors That Influence Therapeutic Success

Factors that adversely affect periodontal regeneration were reviewed at the 1996 World Workshop in Periodontics and 2003 Workshop on Contemporary Science in Clinical Periodontics.[230,231] A number of factors have been implicated or shown to adversely influence periodontal regenerative therapy. These include:

- *Poor plaque control/compliance*: Classical studies of poor plaque control and poor postoperative recall compliance have indicated that much of the therapeutic gain from periodontal surgery will deteriorate over time.[232–236] This response also is observed in GTR regenerated sites.[237–239] Progressive deterioration and a greater incidence of infection with putative periodontal pathogens (*Porphyromonas gingivalis, Prevotella intermedia, and Actinobacillus actinomycetemcomitans*) were more prevalent in patients with poor plaque control and compliance as compared with those with excellent plaque control and maintenance.[240] Furcation repairs also respond similarly, with deterioration for patients with poor plaque control and compliance, and increased stability in patients exhibiting the converse behavior.[241] The difficulty is that patient compliance is hard to maintain.[242–244] Motivating patients to remain highly enthusiastic about oral hygiene and to be compliant with periodontal maintenance is difficult but extremely important (see Chapter 13).

- *Smoking*: Smoking is a major risk factor not only for disease progression, but also for adverse therapeutic outcomes.[245–247] Not only has smoking been implicated as having a detrimental effect on periodontal wound healing after surgical procedures,[248–250] it also has been linked to impaired healing response to GTR procedures in both intrabony defects and furcation repairs.[251]

- *Tooth/Defect factors*: Therapeutic success is influence by the tooth's importance in the prosthetic rehabilitation, its endodontic status, and the defect characteristics.

- The critical question to be addressed is whether the involved tooth is strategically important in the final restorative plan.[252] If not, the procedure may not be justified because of its technical difficulty and expense, potential postsurgical complications, and the difficulty in obtaining excellent patient oral hygiene and compliance.

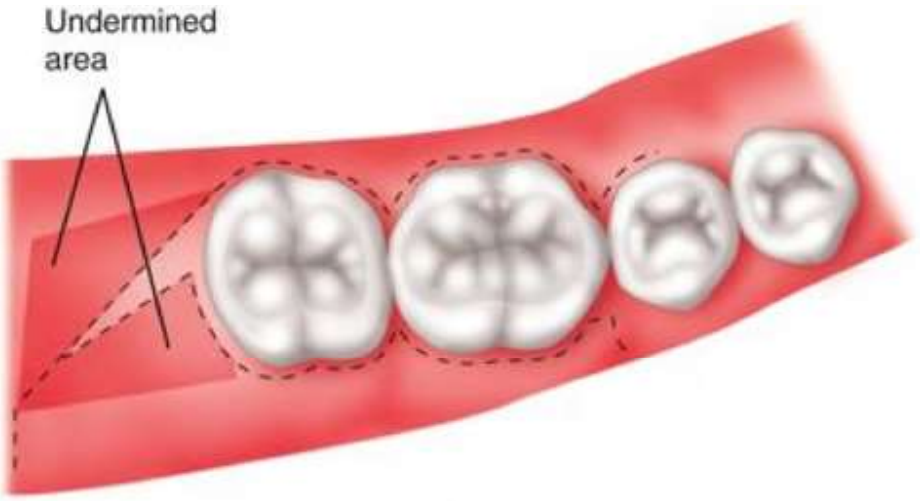
- Once a tooth is deemed essential, it is important to assess its endodontic status. Frequently, chronic endodontic-periodontal defects have the same appearance as an advanced intrabony defect. Treatment of the periodontal component of an endodontic-periodontal defect without first addressing the endodontic component will result in failure.[253,254] The chronicity of the endodontic-periodontal infection may be more important in predicting the outcome of regenerative procedures. Teeth with adequate endodontic therapy appear to respond to regenerative therapy in a way similar to vital teeth without pulpal pathology. Given the expense for endodontic treatment, periodontal regenerative procedures, crown buildup, and the crown strategic extraction and possible replacement with a prosthesis or a dental implant should be considered.

- Characteristics of the defect, such as the overall defect depth, width, and number of walls, can influence clinical outcome in response to regenerative surgery.[50,52,54,55,255] Studies have consistently shown that an increased depth of the defect is correlated with increased improvement in clinical attachment level and probingdepth.[52,55] Therefore, a 7-mm-deep intrabony defect can be expected to demonstrate a greater percent defect fill, greater defect resolution, and greater clinical attachment gain than a 3-mm-deep defect. Conversely, an increased width of the bony defect has been correlated with decreased bone fill and clinical healing response. Defects with more acute angles at the base of the defect (i.e., a steeper vertical inclination of the bony walls) have greater regenerative potential than defects with less acute angles at the base. Lastly, intrabony defects characterized by three- or three- and two-walled configurations will generally respond more positively to regenerative procedures.[235,236,256,257] Barring early reports on the use of iliac and autologous grafts, current regenerative approaches have not been consistently successful in regenerating one- or zero-walled defects. It is likely that defects with a greater number of bony walls have better regenerative potential because of the increased area for influx of bone-forming cells into the defect.

- *Surgical management*: As with any surgical procedure, flap management and wound stability are important (Fig. 25-8). In the regenerative management of intrabony defects, it is important to ascertain presurgically whether there is sufficient keratinized tissue to allow complete tissue coverage of the defect. Surgical flap design should be such that after sulcular or inverse bevel incisions, buccal and lingual full-thickness flaps are reflected extending to at least one to three teeth mesially and distally to the treated tooth. In the case of a missing proximal tooth, the flap should be extended at least 5 to 10 mm proximal to assure adequate visualization of the defect. Visualization often can be enhanced with the placement of vertical releasing incisions. In addition, these incisions can permit

the coronal positioning of the flap. Care should be taken to preserve as much of the keratinized gingiva as possible. In many cases, sulcular incisions are used to maintain the entire zone of keratinized tissue and to ensure complete coverage of any grafts or regenerative membranes that are placed. Interdental tissues should be preserved in their entirety so that flap margins in this region can be coapted to prevent graft or membrane exposure during healing. After flap reflection, it is important to remove all granulation tissues associated with the defect and to thoroughly root plane the surfaces adjacent to the defect. Root defects, such as severe irregularities, cemental pearls, or cementoenamel projections, must be corrected with odontoplasty. After evaluation of the defect, root conditioning may be performed. Regenerative materials may be placed and a GTR membrane applied, if desired. It is important to have good tension-free surgical closure over the defect after suturing and for the wound to remain clinically closed throughout healing. Studies have implicated poor regenerative response because of surgical exposure and infection of membranes used in GTR procedures. These problems were prevalent with nonresorbable membranes; however, current resorbable membranes are more tissue-compatible and it is easier to maintain good tissue coverage over the GTR membrane.

Figure 25-8.



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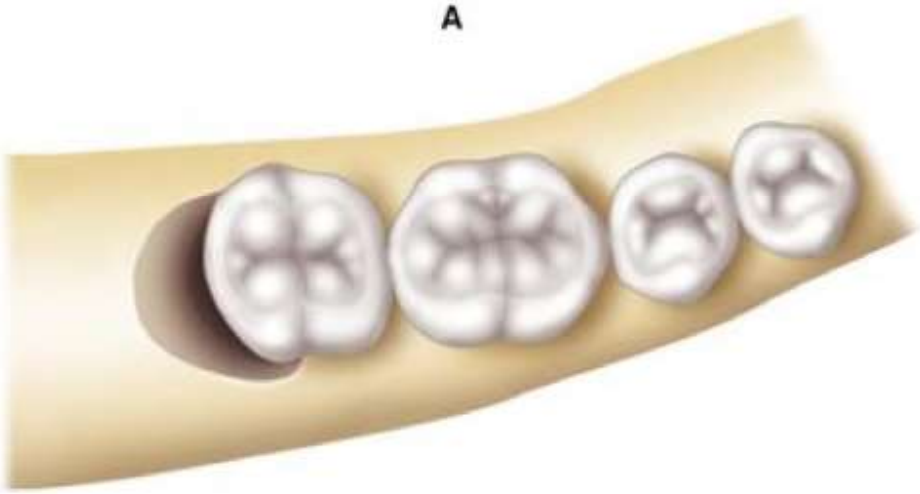

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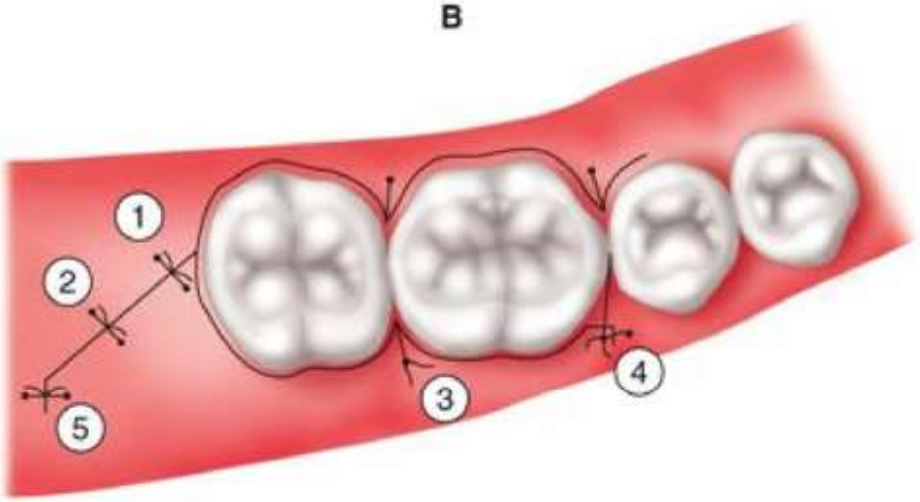


C

Flap management and suturing sequence for periodontal regeneration. Good flap design is essential for visualizing and debriding the osseous defect. In areas of redundant tissues, such as over an edentulous area, the soft tissue may be thinned by undermining. It is essential to achieve tension-free primary closure, which can be accomplished with surgical extension and vertical releasing incisions when needed. Vertical releasing incision should be at least one tooth away from the regeneration site (**A** and **B**). After debridement, the defect is managed and sutured in the sequence as numbered (**C**). The suturing sequence should start at the regeneration site and continue away from the defect area. This ensures good closure over the site of the defect.

CONCLUSION

Over the last three decades, the periodontal literature has been filled with numerous reports related to periodontal regeneration. This therapeutic goal, although ideal, is difficult to achieve. A variety of graft materials and regenerative strategies are currently available; however, they all have limitations. The surgical procedures can be technically demanding, and when successful results are achieved, the maintenance of positive results is highly dependent on patients' oral hygiene habits and compliance with periodontal maintenance. Despite all these difficulties, periodontal regeneration is a clinical possibility that can be offered to patients. The clinician must carefully evaluate the various regenerative and reparative approaches, and then decide which technique may result in the best clinical outcome. With the advent of new regenerative approaches, such as biological modifiers like EMD and growth factors, we must critically evaluate how they may improve our ability to regenerate periodontal defects.

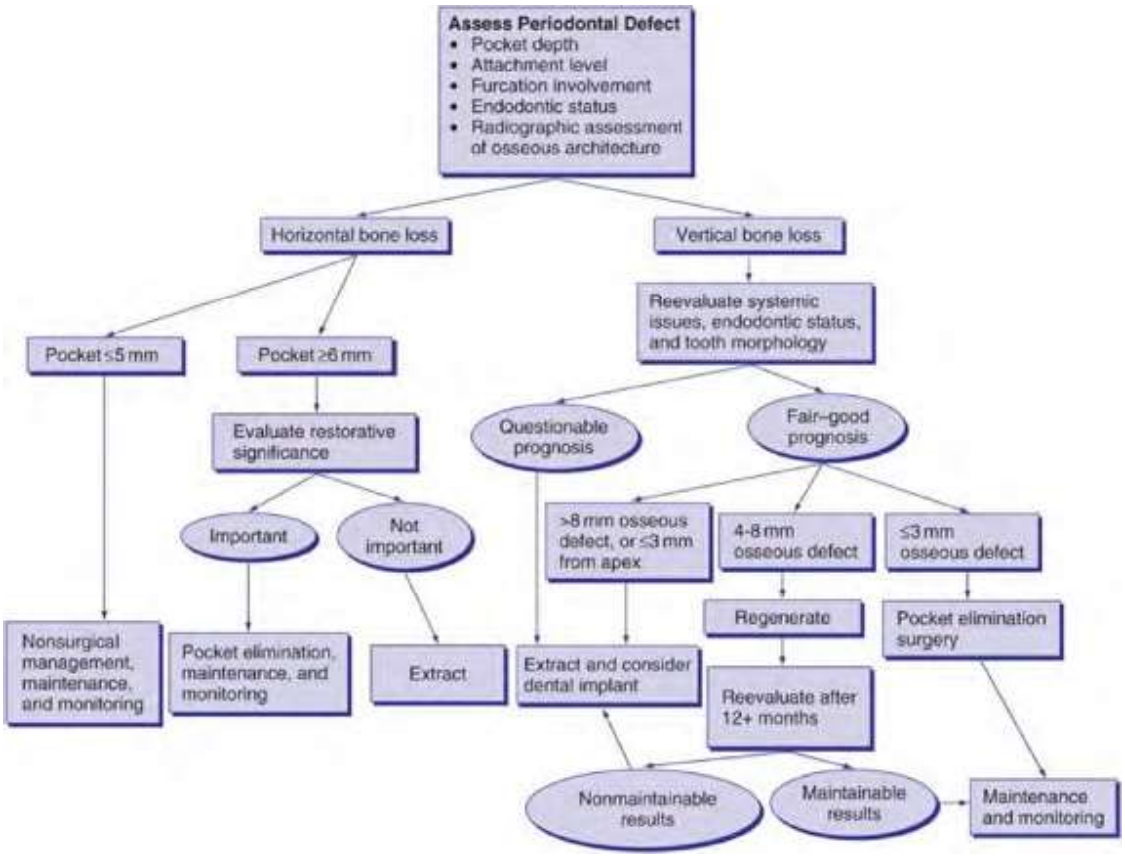
Treatment planning in periodontics also has changed dramatically in the last decade because of the acceptance of dental implants as a viable long-term option for replacing missing teeth. With the increased predictability of implants, the question arises as to when to treat severe periodontal defects with regenerative or other procedures and when to perform strategic extraction in preparation for implant placement. Sometimes the best management of a periodontal defect may be extraction in lieu of periodontal regeneration or when regenerative efforts have been unsuccessful. Extraction would minimize further bone loss and provide the maximum volume of bone at the future implant healing site. This paradigm shift has complicated our views about regeneration. With dental implants as a viable alternative, we may need to redefine periodontal prognosis and consider strategic extraction more often. Heroic regenerative procedures may be contraindicated when extraction and implant placement is considered more predictable and cost effective.

A clinical decision tree is provided to help guide the clinician in deciding the appropriate situations for selecting regenerative procedures over other therapeutic approaches (Fig. 25-9). As with any guidelines, these are intended as a roadmap rather than as a strict set of rules. Clinicians are strongly advised to stay current with changes in the field of regeneration, as well as in other aspects of periodontics and

implantology. As periodontics evolves, the clinical decision tree may need to be modified to accommodate for advances in science and technology.

Periodontal regeneration continues to be one of the primary therapeutic approaches toward the management of periodontal defects. Although evidence suggests that current regenerative techniques can lead to periodontal regeneration, the use of GTR and biological modifiers can enhance these results. The crucial challenge for the clinician is to critically assess whether a periodontal defect can be corrected with a regenerative approach or whether it would be better managed with other treatment options.

Figure 25-9.



Clinical decision tree for the management of advanced periodontal defects.

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