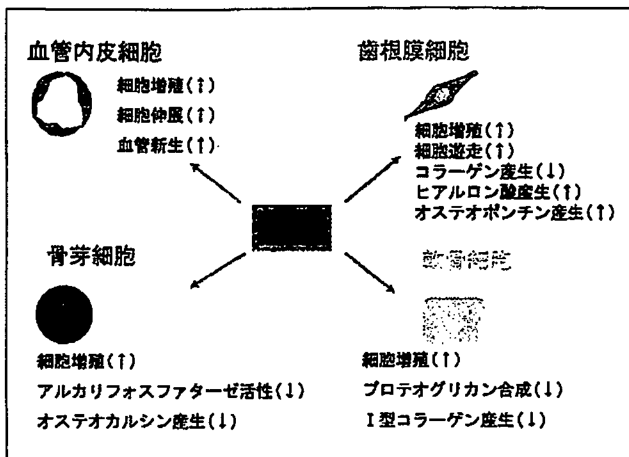


図4　FGF-2の生物学的活性の一例

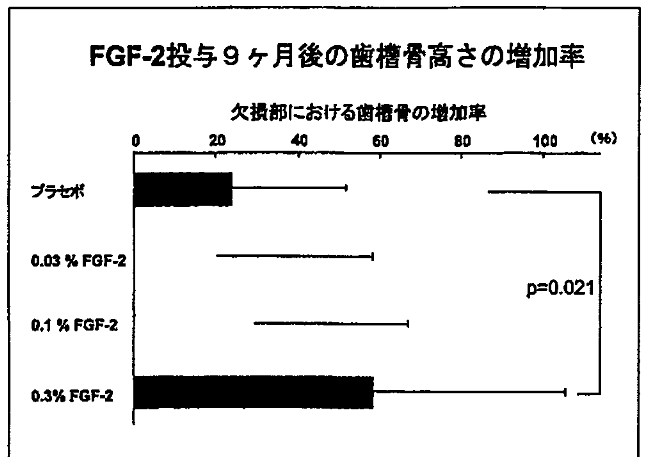


図５　FGF-2投与によりレントゲン的に確認された％歯槽骨再生量（文献７より改変）

材料（medical device）として米国食品医薬局（FDA）の承認を受け、現在、GEM21S®の名で米国での販売が開始されている（図２）。

## 2．FGF-2とは

FGFは、脳および下垂体組織において見出された線維芽細胞の増殖を促進する活性を有するタンパク質であり、その後次々と類似の構造を持つタンパクが発見された結果、現在ではFGF-1～-23からなるファミリーを形成している。FGF-2（以前はbFGFとも略されていた）は、線維芽細胞のみならず血管内皮細胞、神経外胚葉系細胞、骨芽細胞、軟骨細胞、血管平滑筋細胞、上皮細胞などの多種類の細胞の増殖を誘導する。さらに、FGF-2は、組織発生の過程での中胚葉誘導、筋細胞の分化、軟骨細胞や骨芽細胞の増殖、細胞外基質産生の制御にも関わっている（図４）。再生医学の分野でFGF-2が注目される理由として、その強力な血管新生促進作用が挙げられる。さらに、FGF-2は未分化間葉系細胞の多分化能を保持させたまま、その細胞増殖を促進する活性を有していることも理由の一つとして挙げられるであろう。FGF-2は、褥瘡性潰瘍等の難治性皮膚潰瘍の治療薬（Fiblast Spray®）として既に臨床応用されている（図２）。また前臨床研究として、難治性骨疾患の治療にもFGF-2を応用しようとする試みがなされている（図２）。

## 3．FGF-2の歯周組織再生誘導効果の検討

我々は、FGF-2が実際に歯周組織再生を促進するか否かを、動物実験により検証してきた[5,6]。現在までに、ビーグル犬の歯槽骨に作製した２・３壁性骨欠損、２級根分岐部病変、自然発症歯周炎における根分岐部病変、およびカニクイザルの歯槽骨に作製した２級根分岐部病変に0.1％～0.4％のFGF-2を局所投与することにより、統計学的に有意な新生骨量、新生骨梁量、新生セメント質量の変化を伴った歯周組織再生がこれら骨欠損部に誘導されてくることを見出した。また、同部位においてシャーピー線維も再現されているのが確認された[5]。しかも、いずれの症例のFGF-2投与側においても、上皮の下方増殖、骨性癒着、歯根吸収等の異常な治癒所見は観察されてはいない。

2001年よりFGF-2の歯周組織再生誘導効果ならびに安全性の検討を目的として、全国の13施設が参加しての第Ⅱ相臨床治験（プラセボを含む用量反応同時対照による二重盲検試験）が展開された。本治

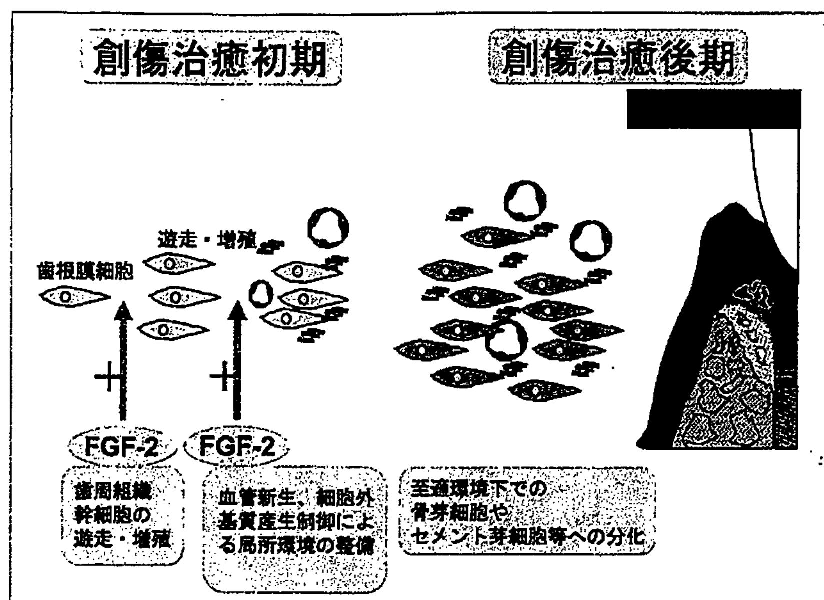


図6　歯周組織再生に対するFGF-2の作用機序（仮説）
FGF-2は、未成熟なヒト歯根膜細胞の遊走・増殖を、その分化能を保持させたままで強く促進する。さらに、FGF-2は創傷治癒における重要な過程である血管新生や、高分子量型ヒアルロン酸やオステオポンチン等の細胞外基質産生を促進し、歯周組織欠損部に「再生」にふさわしい局所環境を創出する。結果として、FGF-2は、歯周組織における創傷治癒の早期に働いて、硬組織形成能を有する未分化な歯根膜細胞の増殖を促進し、その数と密度を骨欠損部で高めることにより、効果的な歯周組織の再生を誘導するものと考えられる。

験ではプラセボ（基剤として用いられた3％ hydroxypropylcelluloseのみ）と、0.03％，0.1％，0.3％ FGF-2含有治験薬を用いて歯周組織再生誘導薬としての有効性と安全性が検討された。その結果、ヒトの2壁性および3壁性歯槽骨欠損に対し、0.3％ FGF-2の局所投与がレントゲン写真上で統計学的に有意な歯槽骨新生を誘導し得ることが確認された（図5）[7]。また、同治験期間中には安全性上問題になるような事例は認められなかった。

FGF-2による歯周組織再生誘導のメカニズムを知る一助として、FGF-2の培養ヒト歯根膜由来細胞（HPDL）に対する作用を、我々は詳細に検討している。その結果、FGF-2は、HPDLの遊走と増殖を促進すること[8]、HPDLからのオステオポンチンやヒアルロン酸等の細胞外基質の産生を高めることを明らかにしている[9, 10]。これらの細胞外基質は歯周組織再生にふさわしい局所環境を創出するのに重要な役割を演じているものと考えられる（図6）。すなわち、歯周組織欠損部において、FGF-2はHPDLを未分化な状態に保ちつつ増殖を促進することにより、治癒の場でのHPDLの細胞密度を増加させ、かつ歯周組織欠損部へのHPDLの遊走を促進し、歯周組織再生過程における初期過程を活性化する。

一方、FGF-2は投与部位における血管新生を促進し細胞外基質の産生を制御することにより歯周組織再生にふさわしい局所環境を整備すると考えられる。このようにして、FGF-2投与部位（すなわち再生を期待する歯周組織欠損部）において、その数を増大させたHPDLがFGF-2により創出された至適環境の中でセメント芽細胞や骨芽細胞への分化を開始し、結果的に歯槽骨、セメント質の新生を含む歯周組織の再生が量的、時間的に促進されるものと考えられる（図6）。

## 4．細胞増殖因子を用いた歯周組織再生治療の将来展望

本項においては、歯周組織再生治療への応用が期待される細胞増殖因子としてPDGFとFGF-2を中心に紹介をさせていただいた。またこれ以外にも、歯周組織再生治療用としては適応がとられていないが、骨形成タンパク-2（bone morphogenetic protein-2: BMP-2）とよばれる因子（厳密には細胞分化因子とよぶべきであろう）と足場材としてウシ由来Ⅰ型コラーゲンを組み合わせた製剤（INFUSE®：GEM21

と同様に medical device に分類されている）が sinus lift や ridge augmentation に対する適応をとり、米国において販売が開始されている。このように、細胞増殖因子を再生医療に応用する試みは、今後も多方面で推進されるものと期待される。先に述べたように GEM21S®の場合には細胞増殖因子（PDGF）と骨伝導性の足場材（β-TCP）を組み合わせた剤型になっている。一方、現在臨床治験が進められている FGF-2製剤に関しては、組織工学における"足場"の概念を現時点では導入していない。これは、FGF-2単独の有効性と安全性をまず明確にすることを目的として臨床治験が行われているためである。しかしながら、次のステップにおいては、FGF-2の基剤により組織工学的な工夫が求められることになるとの展望を抱いている。すなわち、(1) 歯周組織再生を期待する空間の保持（スペースメイキング）能力を有する、(2) 適度の賦形性を有する、(3) 骨伝導性を有する、(4) Drug delivery system の機能を有する、等の機能を有する FGF-2用足場材（著者らは intelligent scaffold とよんでいる）が開発されることが期待される。このように、細胞増殖因子を用いた歯周組織再生治療は、大きな可能性を秘めた治療法であるが、現時点では依然として新規な治療法であることも事実である。適応症を吟味し、その有効性と安全性を真摯に評価することで、同療法が正しく育成されることが強く望まれる。

本項で記した細胞増殖因子を用いた歯周組織再生治療は、GTR 法やエナメルマトリクスタンパクを用いた再生治療法と同じく、患歯の歯根膜組織に存在する「歯周組織幹細胞」を幹細胞源として用いている。今後は、骨髄や脂肪組織等に由来する間葉系幹細胞を歯周組織欠損部へ移入する治療法も新たな治療オプションとして開発が進むものと期待される。歯周組織再生治療は今後も進歩と進化を続ける歯科治療分野といえるであろう。

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# The use of biologic mediators and tissue engineering in dentistry

RICHARD T. KAO, SHINYA MURAKAMI & O. ROSS BEIRNE

Historically, periodontal and maxillofacial regeneration research has focused on the quest for a 'magic filler' material. This search has led to the development of techniques utilizing autologous bone and bone marrow, allografts, xenografts and various man-made bone substitutes. Although these techniques have had limited success, the need for a more effective regenerative approach resulted in the development of procedures that utilize biological mediators and tissue-engineering techniques. Early experimental approaches with biological mediators consisting of growth factors and morphogens were encouraging. With the use of recombinant DNA technology, some of these growth factors and morphogens have been produced in adequate quantities for clinical application. With their introduction, tissue engineering, a relatively new field of reconstructive biology that utilizes mechanical, cellular and biologic mediators to facilitate reconstruction/regeneration of a particular tissue, is being introduced to dentistry.

The concept of tissue engineering in periodontics began with guided tissue regeneration, a mechanical approach utilizing nonresorbable membranes to regenerate periodontal defects. In dental implantology, guided bone regeneration membranes, with or without mechanical support, are used for bone augmentation of the proposed implant placement site. With the availability of partially purified protein mixture from developing teeth and growth factors – morphogens – from recombinant technology, a new era, whereby biologic mediators can be used for periodontal and maxillofacial regeneration, is approaching. The advantage of recombinant growth factors is that, for the first time, the tissue engineering device is of consistent quality and variations in the regenerative response are limited to the individual's healing response and surgical techniques.

This article reviews how tissue engineering has advanced and discusses its impact on the clinical management of periodontal, maxillofacial and osseous defects in preparation for implant placement. This review focuses on the various components of tissue engineering and how it may contribute to the improvement of surgical outcomes. Furthermore, the review clearly defines concepts that are still in the experimental phase of development vs. clinical applications of tissue engineering. This understanding will aid clinicians, as well as dental researchers, to comprehend and appreciate the potential of tissue engineering in today's ever-expanding periodontal oral plastic surgery procedures.

## Tissue engineering

In wound healing, the natural healing process usually results in tissue scarring or repair. Using tissue engineering, the wound healing process is manipulated so that tissue regeneration occurs (93). This manipulation usually involves one or more of the following three key elements: the signaling molecules; scaffold or supporting matrices; and cells (Fig. 1).

Early clinical examples involving tissue engineering principles include the use of bone allografts and autologous platelet-rich plasma. Investigations, which will be discussed later in this paper, reported that the success rates with these materials were inconsistent. With the development of recombinant growth factors and morphogens, and the use of synthetic scaffolds, the level of success has improved. Once considered

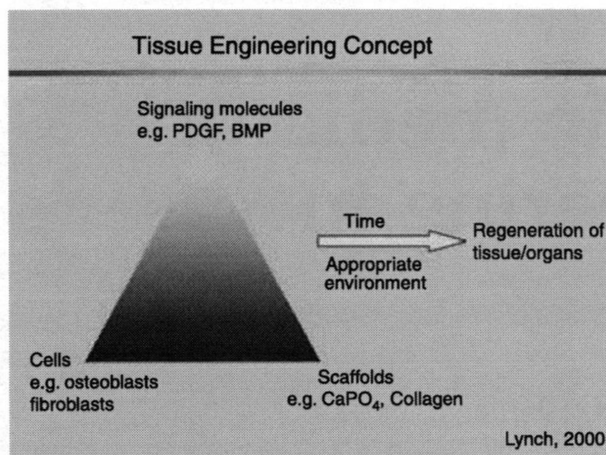


**Fig. 1. Tissue engineering is the manipulation of one or more of the three elements: signaling molecules, scaffolds, or cells. Modified from Lynch (93). BMP, bone morphogenetic protein; FGF-2, fibroblast growth factor-2; PDGF, platelet-derived growth factor; PDL, periodontal ligament.**

experimental in nature, tissue engineering is now clinically applicable with two commercially available tissue engineering systems that involve the use of platelet-derived growth factor-BB tricalcium phosphate (GEM 21®; Osteohealth, Shirley, NY, USA) and bone morphogenetic protein type I collagen sponge (INFUSE®; Medtronic Sonfamore Danek, Memphis, TN, USA). The development of a third promising system utilizing basic fibroblast growth factor-2 is completing multicenter clinical trials.

As tissue engineering approaches are likely to improve clinical results, clinicians need to understand the biology and clinical parameters/limitations of these techniques. In the following sections, each of the three key elements of tissue engineering and how they are applied to the spectrum of periodontal and other orofacial surgery procedures are reviewed.

# Signaling molecules

Since the late 1990s, tissue engineering research has focused on two main approaches involving preparations containing biological mediators to selectively enhance the cells that populate the periodontal wound. The first approach utilized semipurified preparations such as enamel matrix derivative and autologous platelet-rich plasma preparations. The second approach utilized recombinant growth factors such as recombinant human platelet-derived growth factor-BB and recombinant human basic fibroblast growth factor, and morphogens such as recombinant human bone morphogenetic protein. The cellular responses to these biologic mediators *in vitro* have been studied and are summarized in Table 1. These biological mediators enhance periodontal and bone regeneration to varying extents and all are commercially available with the exception of platelet-rich plasma, which is prepared prior to usage.

## Platelet-rich plasma preparation

The use of platelet-rich plasma as a source of growth factors in bone and periodontal regeneration has been proposed (94). In this approach, autologous blood is drawn and separated into three fractions: platelet-poor plasma (fibrin glue or adhesive); platelet-rich plasma; and red blood cells. Platelets are enriched by 338% in the platelet-rich plasma preparation, and the concentrations of platelet-derived growth factor and transforming growth factor-beta1 in platelet-rich plasma have been reported to be 41.1 and 45.9 ng/ml, respectively (87). Monoclonal antibodies have identified the presence of platelet-derived growth factor, insulin-like growth factor and transforming growth factor-beta in the cytoplasmic granules of platelets. Recently, further detailed analysis of platelet-rich plasma indicated the additional presence of basic fibroblast growth factor-2, epidermal growth factor, and vascular endothelial growth factor (28). This mixture of growth factors in platelet-rich plasma putatively stimulates the proliferation of fibroblasts and periodontal ligament cells, extracellular matrix formation and neovascularization. Additionally, platelet-rich plasma may suppress cytokine release and limit inflammation, thereby promoting tissue regeneration (28). Platelet-rich plasma also contains a high concentration of fibrinogen. In clinical use, calcium and thrombin are added to the platelet-rich plasma 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 to these growth factors (6, 7, 94, 135), it is questionable whether the concentrations used are adequate to elicit clinically measurable results. The level of platelet-derived growth factor is 3000-fold less than the concentration needed for periodontal regeneration (67). One possibility is that the enhanced healing is a result of a synergistic effect between the various growth factors found in the mixture, in combination with the anti-inflammatory effect of platelet-rich plasma (28). Alternatively, the accelerated epithelial healing may be a result of the presence of a fibrin clot, which stabilizes the early

**Table 1.** *In vitro* effects of growth factors on periodontal ligament cells and osteoblasts*

| | Platelet-derived growth factor | Fibroblast growth factor-2 | Bone morphogenetic proteins | Enamel matrix derivative | Transforming growth factor-beta | Insulin-like growth factor-1, 2 |
|---|---|---|---|---|---|---|
| Periodontal ligament cells | | | | | | |
| Cell proliferation | ++ | +++ | ++ | ++ | – | + |
| Chemotaxis | ++ | +++ | + | ++ | 0 | ++ |
| Collagen synthesis | + | – | + | + | + | + |
| Protein synthesis | + | + | + | + | + | + |
| Matrix gene expression | ++ | ++/– | ? | + | + | + |
| Cementoblasts | | | | | | |
| Cell proliferation | +++ | ? | – | ++ | ++ | ++ |
| Chemotaxis | ++ | ? | ? | ? | ? | ? |
| Collagen synthesis | + | ? | ++ | ++ | + | + |
| Protein synthesis | + | ? | ++ | ++ | + | + |
| Matrix gene expression | +/– | ? | ++ | ++/- | +/– | +/– |
| Osteoblasts | | | | | | |
| Cell proliferation | ++ | +++ | 0 | ++ | +++ | ++ |
| Chemotaxis | +++ | +++ | + | ++ | +++ | + |
| Collagen synthesis | 0 | ++ | 0 | + | ++ | + |
| Protein synthesis | 0 | + | ND | + | +/– | 0 |
| Matrix gene expression | +/– | ++/– | ++ | ++/– | ++ | ++ |
| Alkaline phosphatase synthesis | 0 | – | ++ | ++ | +/– | 0 |

–, inhibition; 0, no effect; +, effect; ?, unknown effect; ND, not determined.
*Adapted and updated from Kao (79).

wound-healing matrix. Unfortunately, this may give the clinician an inaccurate impression that bony and periodontal regeneration follows a similar course of rapid healing. Although ineffective for periodontal regeneration (27, 172), platelet-rich plasma is used to stabilize graft materials for implant-site augmentation and appears to enhance early soft-tissue healing.

## Enamel matrix derivative

Enamel matrix derivative harvested from developing porcine teeth has recently been reported to induce periodontal regeneration (139, 174). The rationale for the mechanism of action is that enamel matrix derivative contains a mixture of low-molecular-weight proteins that stimulate cell growth and the

differentiation of mesenchymal cells, including osteoblasts (49, 53, 54, 72, 120). Although there were initial concerns about the poorly characterized nature of this preparation, recent reports suggest that the mixture may work synergistically on multiple levels to enhance periodontal regeneration. Enamel matrix derivative has been shown to contain transforming growth factor-beta (104) and to stimulate bone morphogenetic protein, transforming growth factor-beta and connective tissue growth factor expression in osteoblastic cells (53, 61, 152). Connective tissue growth factor is a downstream mediator of transforming growth factor-beta and promotes osteoblast proliferation and development (36, 61, 155). Furthermore, enamel matrix derivative stimulates angiogenesis directly by stimulating endothelial cell proliferation and chemotaxis, and stimulates vascular endothelial cell growth factor production by periodontal ligament cells (134). When applied to root surfaces, the proteins are absorbed into the hydroxyapatite and collagen fibers of the root surface, where they induce cementum formation followed by periodontal regeneration.

*In vitro* studies indicate that enamel matrix derivative may influence the cellular activities of the various cell types in the periodontium. When periodontal ligament cells are exposed to enamel matrix derivative, the cells exhibit enhanced protein production, cell proliferation and the ability to promote mineral nodule formation (22, 39). Cementoblasts and osteoblasts in cell culture treated with enamel matrix derivative show increased cell proliferation, altered gene expression of osteocalcin and osteopontin, and inhibited mineral nodule formation (159). Understanding how cells respond to enamel matrix derivative may elucidate how biomimetic agents work in general. It is only through this understanding that there can be more predictable clinical therapy.

In an animal study, experimental dehiscence defects were created through bilateral removal of the alveolar bone, periodontal ligament and cementum (51). Prior to repositioning the flap, one side was treated with acid-etching and enamel matrix derivative, while the control side was acid etched only. After 8 weeks, histological analyses of the specimens indicated that the enamel matrix derivative-treated side generally showed no gingival recession or formation of a long junctional epithelium and 60–70% of the surface was covered with regenerated acellular cementum. The control sites displayed gingival recession with regeneration on 10% of the surfaces.

## Growth factors for biomimicry

Growth factors are naturally occurring proteins that regulate various aspects of cell growth and development (90, 158). Recently, several growth factors have been identified and characterized, some of which are found in the bone matrix. During wound healing, these growth factors modulate cell proliferation, migration, extracellular matrix formation and other cellular functions. Additionally, some growth factors may also function as cell differentiation factors. In periodontal regeneration, much of the focus has been on platelet-derived growth factor and basic fibroblast growth factor-2.

Most of our information about growth factors comes from cell culture experiments. Prior to 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, etc.), cell proliferation and function, extracellular matrix formation and phenotypic expression were studied (Table 1) (79, 93). With limited supplies, it was not until the advent of recombinant biotechnology that these growth factors were available for use in experimental animal models, which has led to clinical trials and use.

Platelet-derived growth factor was one of the first growth factors studied for its effect on wound healing because it is a potent mitogenic and chemotactic factor for mesenchymal cells in cell culture. Utilizing the information from these cell biology experiments, platelet-derived growth factor and insulin-like growth factor-1 were topically applied to periodontally diseased root surfaces in beagle dogs (91, 92). Substantial amounts of new bone, cementum and periodontal ligament were present after 2 weeks. The results of this study were subsequently confirmed in three other studies utilizing beagles and experimentally induced periodontitis in nonhuman primates (40, 43, 129). Clinical application of platelet-derived growth factor will be described in subsequent sections of this review.

Basic fibroblast growth factor-2 is another growth factor that has been well studied. Fibroblast growth factor-2 is a basic protein originally isolated from hypophysis with an isoelectric point of 9.6 and a molecular weight of 17,000. This growth factor is one of most potent mitogens for cells of mesodermal and neuroectodermal origin and induces angiogenesis (45). Additionally, it is involved in regulating the proliferation and differentiation of a variety of cell types including fibroblasts, vascular endothelial cells, vascular smooth muscle cells, and neuroectodermal,

osteoblast, cartilage, epidermal and periodontal ligament cells (88, 153). In addition, fibroblast growth factor-2 plays important roles during the processes of tissue regeneration and wound healing (44, 111, 123, 173). Fibroblast growth factor-2 is gaining the attention of researchers as a result of its ability to promote mesenchymal cell proliferation and yet still maintain the multipotent properties of these cells (156).

In medicine, fibroblast growth factor-2 has been used in as a therapeutic agent for intractable ulcers for more than 4 years. This has led to the evaluation of fibroblast growth factor-2 as a potential enhancer for periodontal regeneration. In two separate studies utilizing the experimental beagle and nonhuman primate (*Macaca fascicularis*) model system, topical application of recombinant fibroblast growth factor-2 was applied to experimental class II furcation defects (103, 154). After 6 and 8 weeks, respectively, statistically significant improved periodontal regeneration in the defect sites was observed based on morphological and histomorphometrical measurements. No instances of epithelial down-growth, ankylosis, or root resorption were observed at any of the fibroblast growth factor-2-treated sites. Investigations into the possible role of fibroblast growth factor-2 in enhancing periodontal regeneration indicates that fibroblast growth factor-2 not only promotes proliferation of periodontal ligament cells (88, 153), but also induces these cells to produce a regenerative environment that would support regeneration and angiogenesis. The cells are induced to produce an extracellular matrix containing hyaluronan, heparan sulfate and ostepontin, which is commonly associated with wound healing (145, 146, 157). These results strongly suggest that fibroblast growth factor-2 may be efficacious in human periodontal regeneration. Clinical trials of fibroblast growth factor-2 will be described in subsequent sections of this review.

The unique advantage of recombinant human platelet-derived growth factor, tricalcium phosphate and recombinant human basic fibroblast growth factor is consistency in their regenerative capacity. Unlike grafting materials and enamel matrix derivative, there is no variability in purification or processing. Variations in the regenerative/healing response are caused by individual healing capability and surgical techniques. Whereas it is not possible to clinically control individual healing capability, more sophisticated surgical techniques and procedures can be developed. Further research is needed to determine if other surgical parameters, such as the use of guided tissue regeneration membrane and root conditioning, in conjunction with these growth factors will improve the regenerative response.

## Morphogens or differentiation factors: bone morphogenetic proteins

Bone morphogenetic proteins are a group of regulatory glycoproteins that are members of the transforming growth factor-beta superfamily. These molecules primarily stimulate differentiation of mesenchymal stem cells into chondroblasts and osteoblasts. At least seven bone morphogenetic proteins have been isolated from bovine and human sources. In the field of periodontal regeneration, much of the research interest has focused on bone morphogenetic protein-2 (OP-2), bone morphogenetic protein-3 (osteogenin) and bone morphogenetic protein-7 (OP-1) (95).

The osteoinductive effect of bone morphogenetic proteins has been characterized using crude protein preparations derived from decalcified bone. When these crude preparations were placed in muscle or subdermal pouches, 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 (freeze-dried bone allograft and demineralized freeze-dried bone allograft) as osteogenic graft materials. However, when the actual concentration of bone morphogenetic proteins in commercial bone preparations was measured, the amount present was quite low. Approximately 10 kg of bovine bone yields only 2 μg of bone morphogenetic protein (137). This has resulted in efforts to purify, identify and characterize bone morphogenetic proteins so that they can be synthetically produced using recombinant DNA technology.

Experiments utilizing crude and recombinant bone morphogenetic proteins have provided insight as to their potential use. Crude preparations of bone morphogenetic protein-2 and bone morphogenetic protein-3 applied in surgically induced furcation defects appeared to stimulate periodontal regeneration (124). Recent studies have utilized recombinant human bone morphogenetic proteins to determine their potential for correcting intrabony, supra-alveolar, furcation and fenestration defects (42, 69, 83, 148, 160, 168). When recombinant human bone morphogenetic protein-2 was used in supra-alveolar periodontal defects, the gains in bone and cementum were 3.5 mm and 1.6 mm, respectively, compared with 0.8 mm and 0.4 mm for

controls (146). Histologic analysis revealed periodontal regeneration with areas of ankylosis. Contrary to these findings, bone morphogenetic protein-7 augmentation resulted in a significant increase in periodontal regeneration without any ankylosis (148). Healing through ankylosis has been a concern, so most of the recent research utilizing recombinant human bone morphogenetic proteins has involved implant site preparation (10, 18, 23, 24, 52, 68, 163, 168).

## Gene therapy

Among the major limitations associated with the use of growth and differentiation factors are their short biological half-lives. Once applied, these factors are subject to proteolytic breakdown and receptor-binding problems and are dependent on the stability of the carrier system. Clinicians should understand that these growth and differentiation factors have biological half-lives of minutes to a few hours. In addition, an interesting phenomenon to consider when using these factors is that the induced events leading to periodontal regeneration or bone formation occur long after the signaling molecules are no longer present.

Gene therapy can be used for extended local delivery of these factors (30). Recently, gene delivery of platelet-derived growth factor was accomplished by the successful transfer of the platelet-derived growth factor gene into the cementoblast and other periodontal cell types (41, 74). These studies demonstrated that gene delivery of platelet-derived growth factor stimulated more cementoblast activity than a single application of recombinant platelet-derived growth factor. In another report, cells associated with periodontal wounds were transduced effectively by the use of gene transfer (180). Sustained platelet-derived growth factor gene transfer on cementum formation in an *ex vivo* ectopic biomineralization model showed that platelet-derived growth factor may be required for mineral neogenesis, but that continuous production may inhibit mineral formation by cementoblasts (8). This suggests that gene transfer can provide a sustained level of growth factors, but may be detrimental towards the continuous progression associated with differentiation or wound healing.

Gene therapy studies utilizing bone morphogenetic proteins have also been performed. Fibroblasts (130) and oral keratinocytes (128) transduced with bone morphogenetic protein-7 induced bone formation and converted to osteoblasts upon implantation *in vivo* (73). The implication is that this strategy may be employed to promote cementogenesis and osteogenesis. Thus, bone morphogenetic protein-7-transduced fibroblasts were used to stimulate repair of alveolar bone wounds and represent the first successful evidence of periodontal tissue engineering employing *ex vivo* gene transfer of bone morphogenetic proteins.

Gene therapy approaches have also been used to study the regulation of bone morphogenetic proteins. Although osteogenic activity can be demonstrated for bone morphogenetic protein vectors, synergistic results may be achievable with the use of complementary osteogenic signals such as vectors transduced with platelet-derived growth factor (41, 74) and bone sialoprotein (50). Furthermore, the timing and magnitude of expression of particular genes can be varied by controlling the inducible bone morphogenetic protein promoter (32) or antagonist-like noggins (75). By doing this, gene regulation can be manipulated to result in a healing response that mimics regeneration.

The use of gene delivery offers a new approach to delivering growth factors. Although our understanding of gene regulation of platelet-derived growth factor and bone morphogenetic protein has improved with experimental gene therapy studies, the safety and efficacy of using gene therapy for regeneration have yet to be evaluated.

# Scaffold or supporting matrices

Supporting matrices for engineering bone and soft tissue have included processed bone allografts, synthetic and natural polymers, synthetic ceramics, bovine type I collagen and calcium sulfate (Table 2). The major roles for the supporting matrices are listed below:

1. To provide physical support for the healing area so that there is no collapse of the surrounding tissue into the wound site. Examples of this would be bone allografts and synthetic ceramics such as tricalcium phosphate.
2. To serve as a barrier to restrict cellular migration in a selective manner. This is best exemplified by the principles of guided tissue regeneration and guided bone regeneration where nonresorbable polytetrafluoroethylene and resorbable polylactate, polyglyolic acid and calcium sulfate are used. The exclusive properties of cell-barrier membranes have been reviewed (79, 113) and will not be addressed here.

**Table 2.** Commercially available scaffold materials potentially available for oral tissue engineering application

| Biomaterial | Commercial name |
|---|---|
| Allografts | |
| Demineralized freeze-dried bone allograft | Distributed under various names by tissue banks |
| Freeze-dried bone allograft | Distributed under various names by tissue banks |
| Xenografts | |
| Anorganic bovine bone | Bio-Oss®, OsteoGraf®, Pep-Gen P-15® |
| Hydroxyapatite | |
| Alloplasts | |
| Tricalcium phosphate | Synthograft® |
| Hydroxyapatite | Periograf®, Osteogen®, ProOsteone® |
| Bioactive glass polymers | PerioGlas®, BioGran® |
| Hard-tissue replacement polymer | Bioplant® |
| Coraline calcium carbonate | Biocoral® |
| Polymers and Collagens | |
| Collagen | Collaplug®, Collacote®, Gelfoam®, Helistat® |
| Poly(lactide-co-polyglycolide) | |
| Methylcellulose | |
| Hyaluronic acid ester | HY® |
| Chitosan | |

3. To serve as a scaffold for cellular migration and proliferation. Examples include collagen matrix. Potentially, this scaffold can be further enhanced by selectively defining the types of cells permitted to attach to and proliferate on this matrix with the additions of adhesins and / or integrins.
4. To potentially serve as a time-release mechanism for signaling molecules.

## Allogenic and alloplastic bone grafting material

The classic approach to orofacial regeneration in the past 30 years has been the use of bone grafts or substitutes to repair periodontal and maxillofacial defects. The literature contains several excellent reviews on the use of autografts, allografts and alloplastic graft materials (37, 79, 80, 113).

Since the mid-1970s, mineralized and demineralized freeze-dried bone allografts have often been the regeneration material of choice. In addition to their availability and putative osteogenic potential, various clinical studies indicate that 2–3 mm of bone fill is possible with freeze-dried bone allograft (98, 100, 131, 143) and demineralized freeze-dried bone allograft (13, 15, 99, 112, 127). However, other studies have questioned the osteogenic potential of bone allografts (13, 14, 144), suggesting that this may vary depending on the bone bank or batch (137, 138), processing procedures utilized and donor characteristics (71, 147, 177, 178). Owing to varying osteoinductive properties, which is not an area that the Federal Drug Administration regulates, growth factors / morphogens with freeze-dried bone allograft / demineralized freeze-dried bone allograft are not commercially available. However, off-label use of this combination is common both in the orthopedic and oral-periodontal surgical procedures.

Alternatively, a variety of xenograft and alloplastic grafting materials are available as scaffolding agents for tissue engineering. Alloplastic bone grafts consist of ceramics, such as hydroxyapatite, porous hydroxyapatite, tricalcium phosphate and biocompatible composite polymers (HTR). Of these allografts, tricalcium phosphate is used in combination with recombinant human platelet-derived growth factor-BB. In the development of recombinant human platelet-derived growth factor-BB for clinical use, the biosynthetic tricalcium phosphate was used because it possesses defined and reproducible properties as required by the Food and Drug Administration. Allografts were not desirable because of their varying osteogenic potential and properties (137). Extensive animal and human studies have demonstrated biocompatibility of the tricalcium phosphate with no reports of adverse reactions (11, 34). This material physically fills bone defects, provides a scaffold for new bone formation and prevents soft tissue collapse into the wound site (34). Clinically, tricalcium phosphate is osteoconductive and supports early healing. Tricalcium phosphate resorbs at varying rates depending on the chemical structure, porosity and particle size. Absorption, release and bioactivity studies indicate that either tricalcium phosphate or calcium sulfate can be an effective carrier for platelet-derived growth factor-BB. Approximately 45% of the adsorbed platelet-derived growth factor-BB was

released after 10 days (11). In clinical studies with recombinant human platelet-derived growth factor-BB, this material resorbed and was replaced with regenerated periodontium. Superficial granules at the soft tissue interface appeared to resorb at a slower rate. Recently, the use of tricalcium phosphate coated with recombinant human growth/differentiation factor-5 was evaluated for its osteoinductive and osteoconductive properties in an experimental rat calvarial critical size defect (116). Histomorphometric results suggest that this proprietary coating of growth factor on tricalcium phosphate achieved superior bone regeneration compared with conventional materials. The results of these latter two studies indicate that the absorption and release kinetics of signaling agents is an area that requires further elucidation if we are to achieve an optimal regenerative response.

## Collagen carriers

Collagen is the main structural protein for tissue support. It also plays an essential role in wound healing by providing a biologic scaffold for cellular activities such as cell attachment, migration and proliferation (169, 170). Collagen has been widely used in tissue engineering for seeding mesenchymal stem cells (18, 77) and incorporation of growth factors (66, 68). Because most collagens are derived from bovine dermal or skeletal tissue, concerns related to the purity, quality, immunogenicity and potential for prion transmission have been raised (63). Recent studies addressed these issues by further purifying bovine collagen and modifying the molecules to remove its antigenic N- and C-terminal telopeptides (66). Two variations of this collagen were produced to create an atelocollagen scaffold where both constructs were effective in supporting recombinant human bone morphogenetic protein-induced bone formation.

## Calcium sulfate

Calcium sulfate is one of the oldest bone graft materials. Early clinical and animal studies indicate that calcium sulfate is biocompatible, degrades over time, is subsequently replaced with regenerated bone and may be used in an infected area with no complications (117). Recently, studies indicate that it also has barrier properties (79, 81, 115), enhances angiogenesis (151) and may be effective as a delivery vehicle for antibiotics as well as for growth factors (12, 26, 126). Rosenblum et al. (126) demonstrated that fibroblast growth factor was observed to be released at a rate directly proportional to the rate of calcium sulfate dissolution. A secondary benefit of calcium sulfate dissolution is a local decrease in pH. An interesting study in the orthopedic literature reported that when an experimental sheep distal femoral cancellous defect was filled with calcium sulfate, increased immunostaining for bone morphogenetic protein-2, bone morphogenetic protein-7, transforming growth factor-beta and platelet-derived growth factor-BB was observed (165). All of these growth factors have been demonstrated to stimulate bone formation and development. Calcium sulfate was found to be a suitable carrier for recombinant human platelet-derived growth factor-BB with a longer release kinetic profile (~16 days) compared with tricalcium phosphate (11). As both materials are resorbable, the current debate centers on whether a longer sustained release of recombinant human platelet-derived growth factor-BB would be more advantageous to periodontal and bone regeneration.

## Other carriers

Bioresorbable polymers of polylactide-co-glycolide and polyglycolic acid have been considered as scaffolding agents for tissue engineering because of their biodegradable and tissue-compatibility properties. Though promising as a carrier for osteogenic factors in animals (102, 162), variable tissue responses have made clinical application of this material problematic. These tissue responses include inflammation, foreign body reaction and local acid accumulation during polymer degradation (82, 86, 101).

## Substrate modification to enhance cell selection

Although still in the experimental phase, many investigations in orthopedics have focused on the cellular response to synthetic peptide-coated biomaterials (55). When coated with various cell-binding peptide sequences of cell adhesins or integrins, the scaffold mimics the extracellular matrix components of bone. This type of scaffold contains specific surface peptides that selectively permit cell binding, osteoblastic phenotypic expression and differentiation. These constructs can be used to direct the regenerative response. Current focus in this area has been on coating scaffolds with arginine-glycine-aspartic acid (RGD) and phyenyla-

lanine-histidine-arginine-arginine-isoleucine-lysine-alanine (FHRRIKA) peptides selected from the cell-binding and putative heparin-binding domains of bone sialoprotein (48, 122). Additionally, these synthetic peptides can potentially be used to enhance osseointegration (9, 46). Although these products are still primarily in the *in vitro* research phase, bone regeneration has been reported in an experimental dog study (114). In this study, deproteinized bone granules were coated with the 15-amino acid oligopeptides corresponding to the bone morphogenetic protein cell receptor I and II-binding domains. When this engineered graft material was used in the beagle alveolar 3-wall defect model, enhanced new bone formation was found. This approach of directing tissue regeneration by selectively permitting the appropriate stem cells to colonize the wound site holds much potential, but requires further investigation to determine whether this approach is clinically applicable.

## Cell therapy

Cell therapy has been recently employed in periodontal surgery. The most common application involves a cell-expansion strategy in an *ex vivo* environment followed by transplantation back into the defect area. Reducing or eliminating gingival recession has been the objective of most of these studies. Reddy used the patient's own connective tissue to improve a cosmetic defect (121). Unfortunately, the explanted material required a substantial volume of donor tissue and the advantage of this approach was not evident. Pini Prato et al. (118, 119) and Hou et al. (65) reported on the use of harvested fibroblasts seeded onto a nonwoven three-dimensional hydroxyapatite-benzyl ester of hyaluronic acid matrix for gingival grafting. Although the grafts healed well, the increased width of the attached gingiva varied. Momose et al. (102) used a similar approach by seeding attached gingival tissue consisting of both epithelium and connective tissue from the retromolar pad onto a collagen/silicone bilayer membrane. After cell expansion, the graft was used as the donor tissue. This group reported that the graft composed of vascular endothelial growth factor, transforming growth factor-delta and transforming growth factor-beta1 improved wound healing. Tissue-banked human fibroblasts cultured on a bioabsorbable polyglactin mesh were used by McGuire & Nunn for gingival grafting procedures (96). The tissue-engineered fibroblasts-polyglactin mesh graft had comparable clinical results with the exception of increased shrinkage compared with gingival autografts and a decreased amount of keratinized tissue (96). When this graft was used in a subepithelial connective tissue graft procedure, the results were comparable to controls utilizing a connective tissue autograft (166). Recently, this fibroblast–polyglactin preparation has been shown to enhance bone regeneration slightly in experimental peri-implant dehiscence defects (150). The convenience of having tissue-banked viable cell grafts suggests that cell therapy may potentially be feasible should the clinical results be superior and/or comparable with current surgical outcomes and if the economics are not prohibitive.

Although much of the work in cell therapy has focused on fibroblast explants, this approach has been successful with other cell types. Human oral mucosa consisting of epidermal and dermal components has also been engineered where it may be potentially used for intraoral grafting (70). In a case report, cultured gingival epithelial sheets were used to treat multiple quadrants in a patient with chronic desquamative gingivitis (110). After grafting, a decreased number of inflammatory cells, no epithelial-connective tissue separation and gains in keratinized tissue were observed. In another study, regeneration was achieved when a cloned and well-characterized cementoblast cell culture, which exhibits phenotypic expression similar to that of cementum cells, was transplanted into a polylactide-co-glycoide acid carrier and placed in rodent bone defects (179). This example suggests that a reasonable tissue-engineering approach would be to augment appropriate cells in conjunction with current commercial kits containing bone morphogenetic protein-collagen or recombinant human platelet-derived growth factor-BB-tricalcium phosphate to further enhance regeneration.

In the field of craniofacial regeneration, this cell-expansion approach has been used to tissue engineer a human-shaped temporomandibular joint (4, 5, 161). Mesenchymal stem cells were isolated from bone marrow and exposed to either chondrogenic or osteogenic supplemented culture medium. The resulting chondrocytes and osteoblasts were then implanted into a molded hydrogel in the shape of the human temporomandibular joint, which was then implanted into immunodeficient mice for 12 weeks. Upon death of the mice, the resulting histological structure was consistent with the anatomy of a mandibular condyle (5).

Although these reports are proof that tissue-engineering principles can be clinically applicable, the basic mechanisms and optimization of therapeutic application needs further study.

## Application of tissue-engineering principles: periodontal regeneration

Periodontal regeneration remains one of the most difficult surgical objectives to achieve. For clinicians, four criteria are essential in establishing that periodontal regeneration has been achieved. The first is that the tissue-engineering approach must be safe with no adverse clinical or tissue reaction. The second is histological proof that there has been coordinated proliferation, differentiation and development of various cell types to form the three tissue types associated with a regenerated periodontal attachment apparatus, namely new cementum, bone and functionally aligned periodontal ligament. Third, clinical correction of the defect must be demonstrable and maintainable. Finally, evidence that the achieved regenerative response is sustainable needs to be ascertained. This section focuses on tissue-engineering approaches that have been attempted to fulfill these criteria.

### Enamel matrix derivative for periodontal regeneration

Enamel matrix derivative has been effective in the treatment of infrabony defects. Histological evidence for enamel matrix derivative-induced periodontal regeneration has been confirmed in a clinical case report (59). A mandibular lateral incisor destined for orthodontic extraction was treated with acid etching and enamel matrix derivative. After 4 months, the tooth was extracted and examined histologically. Regenerated cementum covered 73% of the defect and regenerated alveolar bone covered 65% of the defect. This histological finding was later confirmed in other case reports (139, 174), whereas new connective tissue attachment was reported in another case series where enamel matrix derivative was used in combination with a bone-derived xenograft (142).

Enamel matrix derivative has been shown to be safe for clinical use (56, 176). Evidence of clinical efficacy was first reported in a multicenter study consisting of 33 patients with at least two defects that were treated using a split-mouth design. The experimental site was treated with acid etching and enamel matrix derivative, whereas the control site was treated with a placebo (60). 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%) enamel matrix derivative-treated teeth, but no bone fill was detected in the controls. The mean radiographic bone fill was greater for the enamel matrix derivative-treated defects that for the control sites (2.7 mm vs. 0.7 mm respectively). Statistically significant improvements were also observed for enamel matrix derivative-treated sites over control sites in mean pocket reduction (3.1 mm vs. 2.3 mm respectively) and mean attachment level gain (2.2 mm vs. 1.7 mm), respectively. These clinical findings have been supported by four additional studies (35, 47, 58, 109), whereas a split-mouth study found no added advantage of using enamel matrix derivative compared with surgical debridement alone (164). Long-term stability of enamel matrix derivative regenerative therapy was reported in a case series that followed 106 enamel matrix derivative-treated defects in 90 patients (57). The data suggest the radiographic bone level, clinical attachment level gain and reduced pocket depth reached the near-maximal response after 1 year and the results remained stable over 5 years. The use of enamel matrix derivative in furcation defects in a limited case series, although encouraging, have not been convincing (3, 25).

There have been four studies comparing the use of enamel matrix derivative alone or in conjunction with other regenerative approaches. When enamel matrix derivative treatment was compared with guided tissue regeneration utilizing bioresorbable membranes, the clinical results were comparable and stable over a 4-year period (140). No significant difference was found when enamel matrix derivative with bioactive glass ceramics was compared with bioactive glass as the sole grafting material (141). Similar results were found when enamel matrix derivative was used in conjunction with anorganic bone graft material (89, 133).

Enamel matrix derivative remains a very intriguing biological mediator. As we start to understand in more detail the mechanism of action of the potpourri of proteins and growth factors, this may strengthen the biological rationale for clinical use of this material. The concern remains whether commercial batches of enamel matrix derivative will be consistent and provide comparable clinical results in all cases. Perhaps the message is that achievement of a maximum regenerative response will require a mixture of biological mediators. With further characterization of

enamel matrix derivative, we may develop a better synergistic blend that will provide optimal results.

## The use of recombinant human platelet-derived growth factor for periodontal regeneration

Histological evidence of periodontal regeneration was first reported in defects in beagle dogs (91, 92). During the development of platelet-derived growth factor for clinical use, recombinant human platelet-derived growth factor was used in conjunction with allogenic bone to correct class II furcations and interproximal intrabony defects on teeth with poor prognosis which are to be extracted (19, 106). Histological evidence of periodontal regeneration was present with excellent furcation fill.

A human clinical trial was conducted using recombinant human platelet-derived growth factor / recombinant human insulin-like growth factor-1 (108). Utilizing a split-mouth design, defects were treated with either a low dose (50 μg / ml) or high dose (150 μg / ml) of recombinant human platelet-derived growth factor / recombinant human insulin-like growth factor-1. After 9 months, the high-dose recombinant human platelet-derived growth factor / recombinant human insulin-like growth factor-1 induced 2.08 mm of new bone and 43.2% defect fill, compared with 0.75 mm vertical bone height and 18.5% bone fill in controls. Low-dose recombinant human platelet-derived growth factor / recombinant human insulin-like growth factor-1 results were statistically similar to those of the controls. Additionally, this study demonstrated that no adverse immunologic or clinical reaction resulted from use of these agents. A primate study examined the regenerative effects of platelet-derived growth factor / insulin-like growth factor-1 individually and in combination (91). Platelet-derived growth factor alone was found to be as effective as the platelet-derived growth factor / insulin-like growth factor-1 combination in producing new attachment after 3 months. No significant effect was found when insulin-like growth factor was used alone. This study suggests that insulin-like growth factor may not be important at the dose level tested.

Subsequently, the effectiveness of 0.3 mg / ml of recombinant human platelet-derived growth factor + tricalcium phosphate (GEM-21®) to improve attachment level gain, bone level and bone volume significantly compared with tricalcium phosphate was demonstrated after 6 months in a multicenter clinical trial (Fig. 2) (108). A subset of these patients was followed-up for 24 months and a representative case series were reported to be stable with increases in radiographic bone fill compared with the end results after 6 months (Fig. 3) (97). A review of these cases indicates that the results are stable. Another case series suggests that recombinant human platelet-derived growth factor with freeze-dried bone allograft can be combined to achieve excellent results in severe periodontal intrabony defects (105).

Recombinant human platelet-derived growth factor in combination with a tricalcium phosphate carrier is now commercially available. Preliminary studies utilizing recombinant human platelet-derived growth factor-tricalcium phosphate suggest that it is easy to use, requires no barrier membranes and produced results comparable to or superior than other regenerative graft materials. The potential for using recombinant human platelet-derived growth factor for regeneration of furcation defects and implant site preparation still needs to be evaluated.

## The use of recombinant human fibroblast growth factor-2 for periodontal regeneration

Beagle and nonhuman primate studies have demonstrated that topical application of fibroblast growth factor-2 into intraosseous defects in alveolar bones induces significant periodontal tissue regeneration. Histological observation revealed new cementum with Sharpey's fibers, new functionally oriented periodontal ligament fibers and new alveolar bone (103, 154). This suggests that topical application of fibroblast growth factor-2 may be efficacious in regeneration of human periodontal tissue that has been destroyed by periodontitis.

A recent randomized controlled double-masked Phase II clinical trial at 13 Japanese dental facilities compared the therapeutic response to varying doses of fibroblast growth factor-2 vs. the control (84). Eighty patients with a two-walled or a three-walled vertical bone defect ≥3 mm from the alveolar crest were randomly divided into four groups: group A (placebo control); group B (0.03%-fibroblast growth factor-2); group C (0.1%-fibroblast growth factor-2); and group D (0.3%-fibroblast growth factor-2). The subjects underwent periodontal surgery during which 200 μl of the investigational drug or placebo carrier was applied to each test site. After 9 months, a significant increase (P = 0.021) in alveolar bone height was demonstrated by standardized radiographs between group A (23.92%) and group D (58.62%)

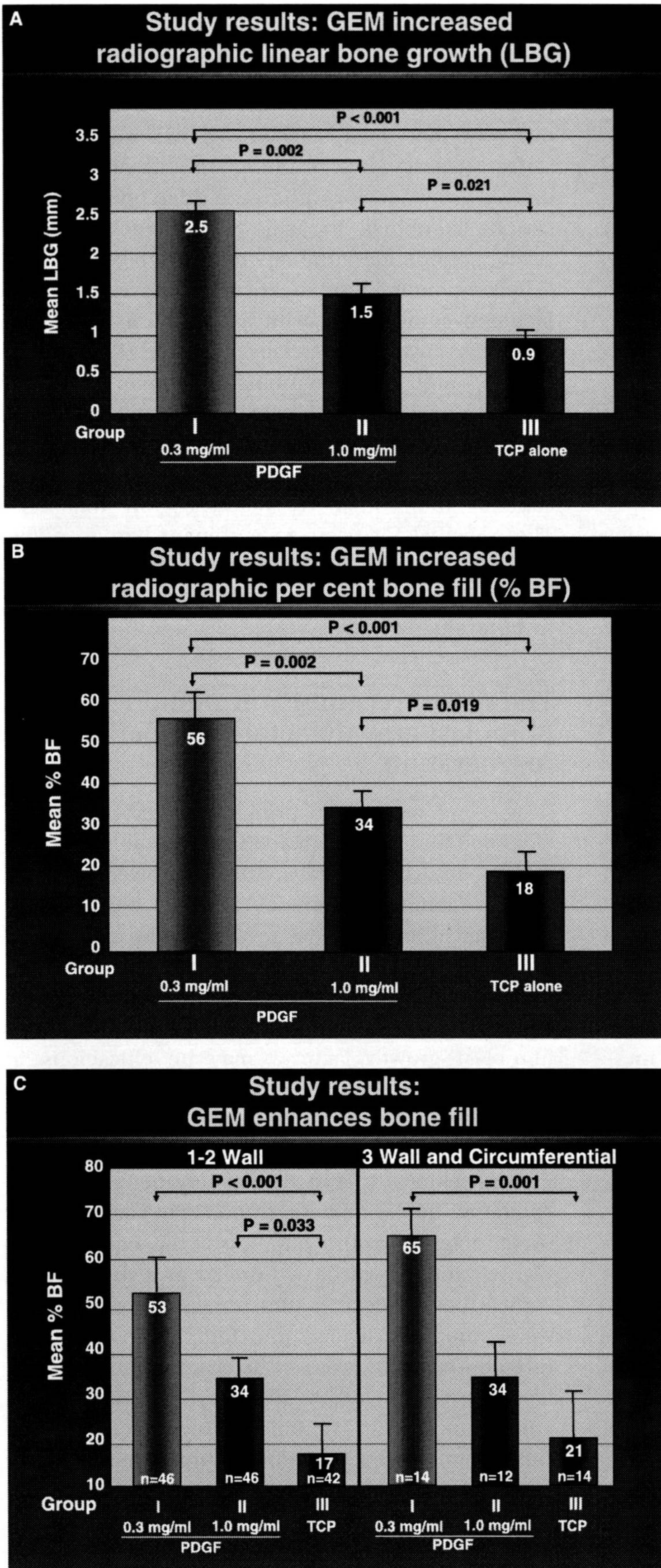


**Fig. 2.** Data from the recombinant human platelet-derived growth factor-tricalcium phosphate pivotal trial (105) indicated that 0.3 mg/ml of recombinant human platelet-derived growth factor resulted in more linear bone growth (A) and percentage of bone fill (B) in all defect types (C) than patients treated with 1.0 mg/ml of recombinant human platelet-derived growth factor or tricalcium phosphate control. (Reproduced with permission of the *J Periodontol*). GEM, GEM 21s, a commercial combination of rhPDGF-BB+beta-tricalcium phosphate; PDGF, platelet-derived growth factor; TCP, tricalcium phosphate.

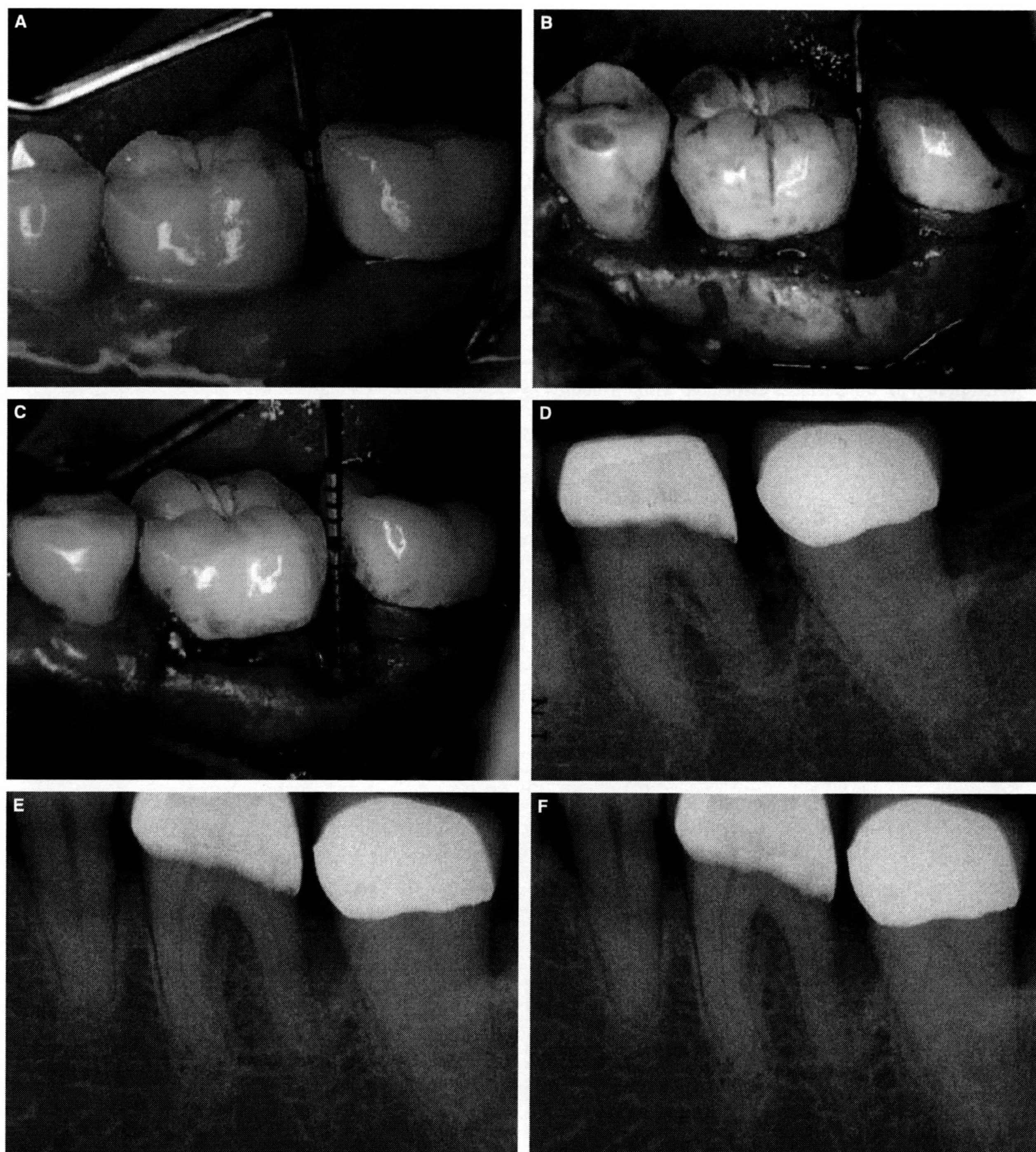


**Fig. 3. A sample case of a patient treated in the pivotal trial (152). Clinical views: pre-surgery (A); defect after surgical debridement (B); and surgical re-entry at 12 months post-surgery showing good bone fill of the circumferential intrabony defect (C). Radiographs taken pre-surgery (D) and at 12 (E) and 60 (F) months post-surgery. The clinical pocket depth was 3 mm.**

(Fig. 4) (84). A typical result from this multicenter trial is depicted in Fig. 5. No adverse effects were observed during the course of this multicenter trial. This finding suggests that topical application of fibroblast growth factor-2 can be efficacious in regenerating periodontal tissue of patients with two-walled or three-walled intrabony defects. This led to a subsequent larger clinical trial that has recently been completed and the results are forthcoming. These trials will provide crucial information regarding the safety and efficacy of utilizing fibroblast growth factor-2 for periodontal regeneration.

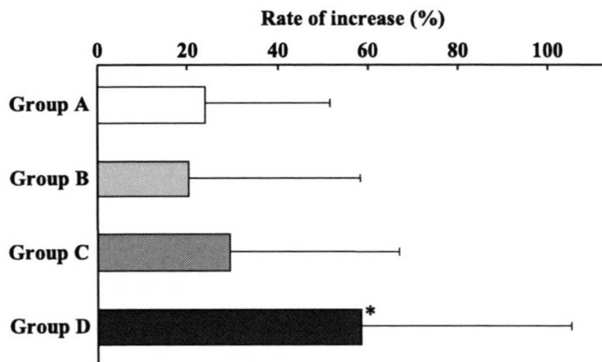


**Fig. 4. Rates of increased alveolar bone heights. Placebo (group A) and recombinant human basic fibroblast growth factor at 0.03% (group B), 0.1% (group C) and 0.3% (group D) were applied to root surfaces associated with infrabony defects. Standardized radiographs were compared pre- and post-treatment (9 months) and the increased bone heights were assessed by five individual oral radiologists for increased alveolar bone heights. No statistical differences were noted with the exception of group D (*P = 0.02).**

# Application of tissue engineering principles: implant site preparation

A second area of focus is applying tissue engineering principles for implant site preparation. Whereas the challenge for periodontal regeneration is the simultaneous regeneration of the three tissues to reconstruct the periodontal apparatus, the challenge for implant site preparation is to regenerate adequate volume of hard and possibly soft tissue. Though much of the focus in this field has centered on the use of recombinant human bone morphogenetic protein, some preliminary data are available which suggest that recombinant human platelet-derived growth factor-BB may be used for this purpose.

## The use of recombinant human platelet-derived growth factor for implant site preparation

Preliminary data are now available which suggest that recombinant human platelet-derived growth factor-BB may be used for implant site preparation. In a standardized dog model, recombinant human platelet-derived growth factor was used in conjunction with an anorganic bone block for vertical ridge augmentation (149). Surgically created defects were grafted with block grafts infused with recombinant human platelet-derived growth factor with and without collagen membrane. Better healing and an increased amount of regenerated bone were observed in sites grafted with recombinant human platelet-derived growth factor-infused block in the absence of a barrier membrane. When these blocks were analyzed utilizing backscattered electron microscopy, the percentage of weight and volume calcium:phosphorus ratios of the regenerated and native bone were found to be similar (125). This suggests that the regenerated bone would have a similar bone-implant interface compared with native bone.

Recently, recombinant human platelet-derived growth factor-BB [from a GEM-21® kit (Osteohealth, Shirley, NY)] was used in conjunction with freeze-dried bone allograft and a barrier membrane to augment both hard and soft tissues simultaneously in preparation for implant placement (29). Following

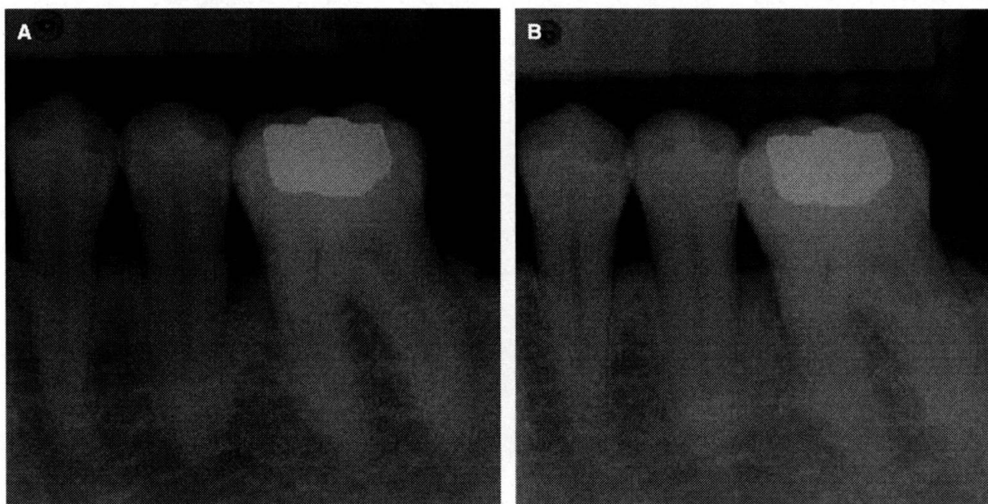


**Fig. 5. (A) Tooth #20 presented with a clinical attachment loss of 10 mm and a pocket depth of 5 mm. The defect was treated with 0.3% recombinant human fibroblast growth factor. (B) After 9 months post-surgically, the clinical attachment loss was 7 mm and pocket depth was 2 mm (photographs courtesy of Dr Matsuyama, Kagoshima University, Kagoshima, Japan).**

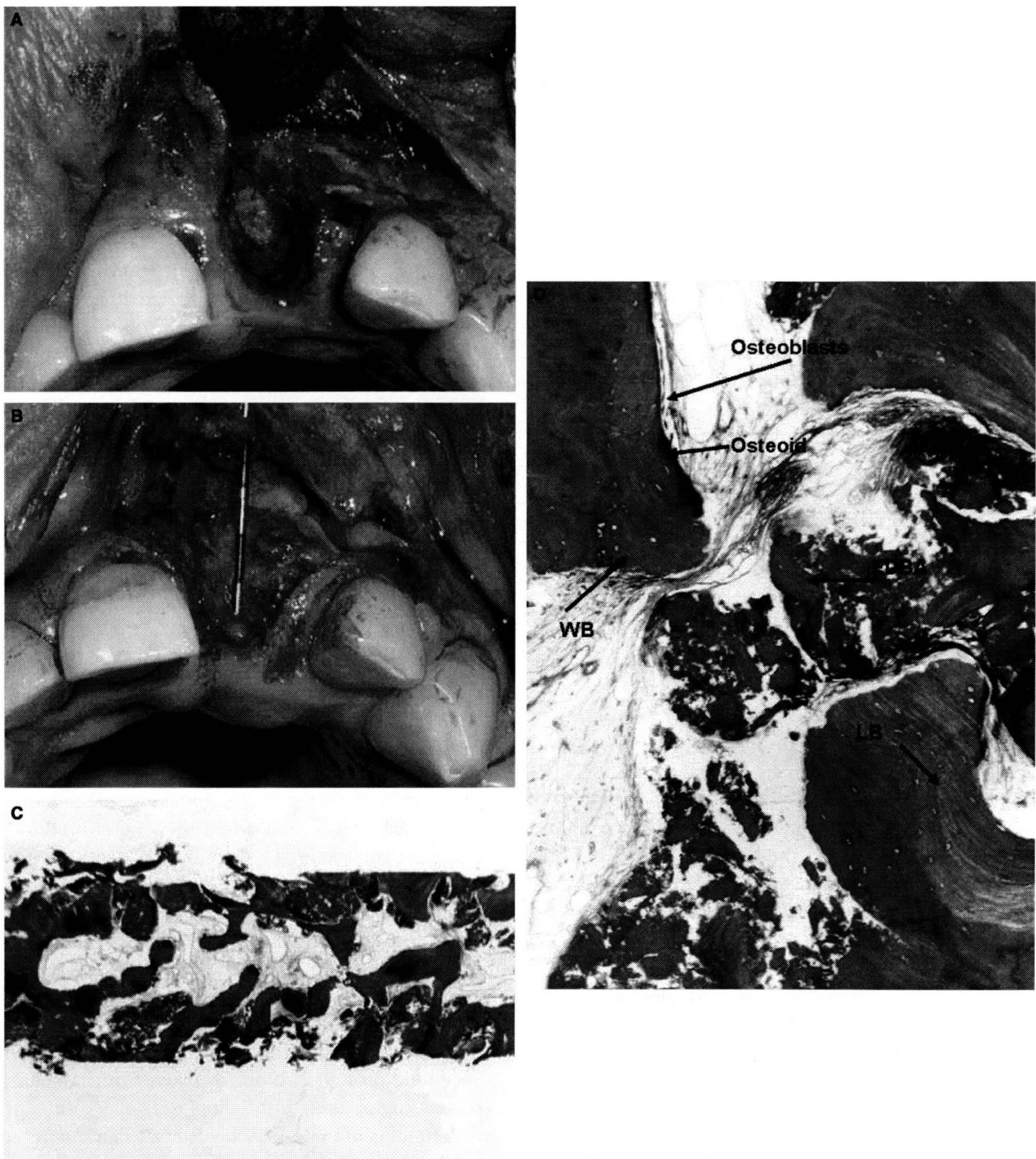


**Fig. 6. The extraction defect for tooth #9 was treated with recombinant human platelet-derived growth factor-freeze-dried bone allograft and a titanium-reinforced membrane (A). On re-entry, the defect was filled with bone (B). Histologic analysis (C, D) showed the new bone formation. FDBA, freeze-dried bone allograft; LB, linear bone formation; WB, woven bone.**

extraction, the bony defect was filled with recombinant human platelet-derived growth factor-freeze-dried bone allograft and covered with expanded polytetrafluoroethylene membrane (Fig. 6A,B). The soft-tissue deficiency was grafted with a pediculated graft from the palate and the soft tissue site was irrigated with recombinant human platelet-derived growth factor prior to closure. After healing, the amount of both bone and soft tissue volume increased. At the time of implant placement, the site was trephined for histological analysis, which revealed the presence of bone regeneration. The

microscopic field contained approximately 48% woven bone and 19% mineralizing osteoid (Fig. 6C,D). This report emphasizes the potential of the use of recombinant human platelet-derived growth factor for simultaneous soft and hard tissue implant site preparation.

## The use of bone morphogenetic protein for implant site preparation

Recent attention has focused on recombinant human bone morphogenetic protein-2 as a replacement for autogenous bone grafts because it reliably induces bone formation and large quantities can be produced using recombinant DNA technology (167). Using various animal species, including nonhuman primates, recombinant human bone morphogenetic protein-2 absorbed into bovine type-1 collagen sponges consistently induced bone at graft sites (16, 38, 107, 160, 171). The recombinant human bone morphogenetic protein-2-soaked collagen sponges reliably induced bone formation in critical sized defects, whereas the defects repaired with sponges without recombinant human bone morphogenetic protein-2 did not fill with bone. Continuity defects and deficient alveolar ridges and maxillary sinuses were successfully reconstructed with recombinant human bone morphogenetic protein-2 (16, 107). Much higher concentrations of recombinant human bone morphogenetic protein-2 were needed to induce bone formation in nonhuman primates than in rodents and rabbits.

Human clinical trials were initiated following the promising results in animals. A feasibility study using an open-label clinical trial demonstrated that recombinant human bone morphogenetic protein-2 soaked into a collagen sponge and placed on the maxillary sinus floor stimulated bone formation (18). A randomized prospective muticenter clinical trial (17) was initiated following the open-label study. Implant survival in the maxillary sinuses augmented with recombinant human bone morphogenetic protein-2 absorbed into collagen sponges was similar to survival of implants placed in sinuses augmented with autografts. A dose–response performed as part of the study showed that the greatest bone induction occurred when 1.5 mg/ml of recombinant human bone morphogenetic protein-2 was used.

Biopsies of the bone induced by the recombinant human bone morphogenetic protein-2 were taken after approximately 7 months of healing. Histological examination revealed that the recombinant human bone morphogenetic protein-2 induced new bone formation. The collagen sponges were no longer present and woven, and lamellar bone filled the grafted sinus floors. The study demonstrated that recombinant human bone morphogenetic protein-2 could induce adequate bone for the placement and functional loading of endosseous implants.

The efficacy of bone morphogenetic protein-2 to augment deficient alveolar ridges has also been evaluated. A preliminary feasibility and safety study demonstrated that recombinant human bone morphogenetic protein-2 on a collagen sponge was safe and might be useful for alveolar ridge augmentation and preservation of bone following tooth extraction (68). The preliminary study was followed by a randomized prospective clinical trial that recruited patients requiring alveolar ridge augmentation following tooth extraction (31). The recombinant human bone morphogenetic protein-2 on the collagen sponge induced more alveolar bone than the collagen sponge alone.

Based on the animal studies and the human clinical trials, the Food and Drug Administration approved recombinant human bone morphogenetic protein-2 (INFUSE®) for use 'as an alternative to autogenous bone graft for sinus augmentations, and for localized alveolar ridge defects associated with extraction sockets' in March 2007 (1). However, the cost of treatment with recombinant human bone morphogenetic protein-2 is high, and less-expensive augmentation materials may be equally as effective as recombinant human bone morphogenetic protein-2 for the augmentation of the maxillary sinuses and ridge preservation following the extraction of teeth (2).

A systematic review of the evidence established predictable augmentation, by alloplasts, allografts, combinations of allografts and alloplasts, and barrier membranes, of maxillary sinus and alveolar ridges for implants (2). Similarly to the biopsies of the sites augmented with recombinant human bone morphogenetic protein-2, biopsies taken from maxillary sinuses augmented with alloplasts/allografts and combinations of materials consistently demonstrated bone induction (132, 136). Figure 7 shows a biopsy taken 6 months after augmentation of a maxillary sinus with freeze-dried cancellous bone allograft (Northwest Tissue Bank, Seattle, WA) combined with hydroxyapatite (Interpore®; Interpore Cross International, Irvine, CA); new bone formation adjacent to the hydroxyapatite and cancellous particles is evident.

Further studies comparing recombinant human bone morphogenetic protein-2 with alloplasts and allographs and combinations of materials are needed to determine if less-expensive materials are equally

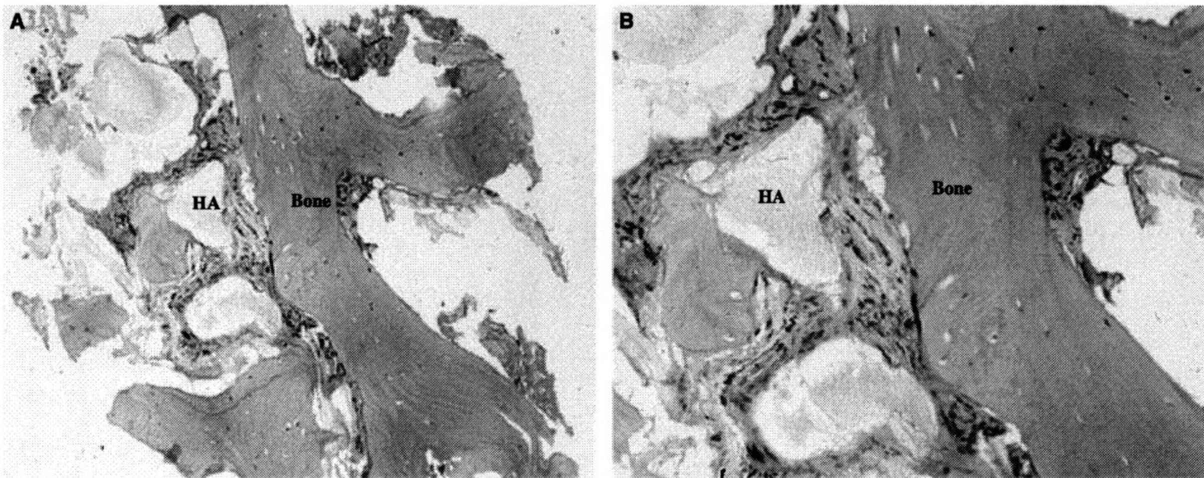


**Fig. 7. (A) A low-powered photomicrograph of a hematoxylin and eosin-stained decalcified biopsy taken 6 months after grafting the sinus floor with hydroxyapatite combined with freeze-dried cancellous bone chips. (B) A higher magnification of the same biopsy. New bone (Bone) can be seen adjacent to the hydroxyapatite / cancellous chip. HA, hydroxyapatite.**

as effective as recombinant human bone morphogenetic protein-2 before routinely using recombinant human bone morphogenetic protein-2 to augment sites with inadequate bone for the placement of endosseous implants.

## Application of tissue-engineering principles: maxillofacial surgical procedures

Tissue regeneration for maxillofacial congenital and acquired defects involves several elements. Cells, scaffolds and growth factors are needed to regenerate a functional replacement for the missing tissue (20, 80). The ideal combination of elements is the current focus of research related to maxillofacial tissue engineering. This discussion will focus on reconstruction of bone defects.

Continuity defects of the mandible frequently develop following trauma or removal of tumors. Large defects are usually repaired using autogenous bone harvested from the iliac crest (80). The autogenous graft is considered the 'gold standard' because it contains three essential elements that are needed to regenerate bone: osteoblasts and osteoprogenitor cells; osteoinductive proteins; and a scaffold of organic and inorganic extracelluar matrix. Tissue-engineered replacements ideally should contain the three elements present in the autogenous bone graft (20, 78). Synthetic scaffolds that replace the structural integrity of the missing bone can be fabricated from several different materials. However, the scaffold must be resorbed and replaced with normal bone because the synthetic scaffold will eventually fatigue and fracture from long-term loading. The scaffolds should be porous to allow adherence of cells and osteogenic proteins as well as vascular invasion into the matrix. Allografts have been used as scaffolds; however, they are not ideal because they have the potential to be immunogenic and to transfer infection (20, 78). The ideal synthetic scaffold should not be immunogenic or have the potential to transfer / support infection. Hollister et al. (64) described an approach to engineer a craniofacial scaffold that used computational design, scaffold fabrication, scaffold structural and mechanical evaluation, and *in vivo* tissue-regeneration tests to develop scaffolds that meet anatomical, load-bearing and tissue-regeneration requirements.

Cells capable of replacing missing tissue are an essential component of engineered tissue replacements. Osteoblasts and mesenchymal stem cells that can differentiate into bone-forming cells are found in bone, bone marrow and periosteum (33, 76, 175). Unlike osteoclasts, which are derived from circulating cells, the osteoprogenitor cells must be present at the graft site or be part of the tissue-engineered graft. Bone marrow aspirate contains adult mesenchymal stem cells that can be induced to transform into osteoblasts and to replace bone (20, 85). Mesenchymal stem cells can be harvested from bone marrow or periosteium and expanded in culture using specific growth media. Stem cells have also been isolated and cultured from dental pulp (85). Large bone defects that no longer have their associated periosteum have few cells capable of transforming into osteoblasts and frequently cannot be repaired without including osteogenic cells in the graft.