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Chapter 28

# Delivery Systems and Role of Growth Factors for Alveolar Bone Regeneration in Dentistry

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## 1. Introduction

Growth factors (GFs) have been investigated for the purpose of alveolar bone regeneration in periodontal, reconstructive and pre-prosthetic surgery, often with a view to rehabilitation with dental implants. Results are promising, and research is currently focusing on developing an effective delivery system capable of ensuring a controlled and localized GF release and activity. In fact, one of the main issues relating to GFs concerns how to control their effects over time so as to guarantee their effective action in the various phases of bone healing. This chapter provides a review of the literature on GFs used for bone regeneration in dentistry, emphasizing the most recent developments relating to local delivery systems.

## 2. Mechanism of action of growth factors (GFs)

Growth factors (GFs) are protein molecules that have a role in controlling biological processes, such as cell growth, proliferation, differentiation and repair. GFs cannot pass through a cell's membrane; they must bind to high-affinity cell receptors in order to take effect. Many GFs stimulate several cell populations, while others are less versatile and specific to a particular cell line.

In dentistry, numerous GFs have been investigated in terms of their effect on hard and soft tissue healing and regeneration.

Whatever the tissue involved, the healing process always involves a series of molecular, biochemical and cellular events that can be grouped into three overlapping phases: inflammation, proliferation, and remodeling.

Inflammation begins spontaneously straight an after injury has occurred and lasts for 1 to 4 days. It is characterized by clotting in the wound, the release of signal molecules to recruit immune cells, and the release of specific enzymes (matrix metalloproteinases, MMPs) that clean the wound. The proliferative phase takes place between 4 and 21 days after wounding, when fibroblasts are stimulated to invade the site of the wound and produce extracellular matrix components. Highly-vascularized granulation tissue is formed and the gap is closed. The final remodeling phase can take up to a year, during which time the immature scar is converted into a stable, less vascularized tissue that exhibits good mechanical proprieties, followed by the growth of regenerated tissue.

GFs have been used in dentistry in all these phases. The most often studied GFs are probably the bone morphogenetic proteins (BMPs), discovered by Urist, who found that protein mixtures obtained from demineralized, lyophilized segments of bone were responsible for bone formation after implanting in rabbit muscle tissue [1].

BMPs are multifunctional cytokines that belong to the transforming growth factor-β (TGF-β) superfamily. They are not only involved in direct ectopic bone formation (hence their name of *bone morphogenetic proteins*), they also modulate several developmental processes, prompting numerous authors to suggest other names: for instance, Reddi suggested that they be should be called *body morphogenetic proteins*, given their extensive roles in various tissues [2].

Over 20 BMPs with various functions have been identified in humans. They have a major role in embryogenesis and in the maintenance and repair of many skeletal and non-skeletal tissues in adults [3]. BMP-1 is actually not considered a member of the BMP family, but a misnamed protein with chordinase and procollagen proteinase activities, implicated in pattern formation during the development of a number of organisms [4]. BMPs are mainly related to bone and cartilage formation, though BMPs 8b, 10 and 15 have no role in these processes, and BMPs 12, 13 and 14 are called cartilage-derived morphogenetic proteins (CDMPs) because they induce chondrogenic phenotypes rather than osteogenesis [2,5], whereas a definite bone-inducing role during bone formation has been observed for BMPs 2, 4, 6, 7 and 9 [6].

BMPs play a pivotal part in skeletal morphogenesis and repair, promoting the differentiation of mesenchymal cells into osteoblasts and inducing new bone formation. BMPs are involved in regulating mesenchymal cell differentiation and proliferation by stimulating intracellular signaling pathways. BMP signals are transmitted by the plasma membrane receptors to the nucleus through multiple signaling pathways that can be divided into two groups, the Smad and non-Smad pathways [3,7]. At the cell surface, BMP ligands bind with BMP receptors, triggering specific intracellular pathways that activate and influence gene transcription. Of the three types of receptor for the TGF-β superfamily, only types I and II appear to have significant roles in BMP binding and signaling. Five type I receptors (ALK1 [Acvrl1], ALK2 [ActRI], ALK3 [BRIa], ALK4 [ActRIb] and ALK6 [(BRIb]), and three type II receptors (BRII, ActRIIa, and ActRIIb) have been identified [8], plus a short form of BRII [9]. Type III TGF-β receptors have

also been shown to have a role in BMP signaling, by mediating epithelial to mesenchymal cell conversion [10].

Canonical Smad-dependent TGF-β first binds to receptors type I and type II, and then signals are transduced to their Smads. Activated Smads form a complex with Smad4 and cross the nuclear membrane into the nucleus, where they regulate the expression of transcriptional factors and transcriptional coactivators that are important in osteoblasts (Dlx5, Runx2 and Osx). It has recently been demonstrated that, following TGF-β induction, the Smad and the p38 MAPK pathways converge on the Runx2 gene to control mesenchymal precursor cell differentiation [11].

As for the isolation of BMPs, after Urist's experiments, BMPs were obtained from the bones of various species, including rabbit, cow and human. Nowadays, BMPs are produced and purified using DNA recombinant technology and essentially two expression systems, in mammalian cells or bacteria [6]. Recombinant human BMP-2 (rhBMP-2) and recombinant human BMP-7 (rhBMP-7) are currently the only proteins in the group to been approved by the US Food and Drug Administration (FDA) for clinical use in humans, which explains why they are clearly the most extensively evaluated BMPs [12].

Another GF of interest in dentistry is the growth and differentiation factor (GDF), the structure of which closely resembles some BMPs, so it could be included in the BMP family. GDF-5 is also known as BMP-14, or cartilage-derived morphogenetic protein 1, because it induces chondrogenic phenotypes rather than osteogenesis [6]. GDF-5 gene mutations give rise to different types of dysplasia and can result in the autosomal recessive syndromes of brachypod in mice and Hunter-Thompson or Grebe-type chondrodysplasia in humans, involving a loss of joints in both humans and mice [13-15]. Francis-West and colleagues [14] showed that GDF-5 can modulate the initial stages of chondrogenesis by increasing cell adhesion, and can increase chondrocyte proliferation in the later stages of skeletogenesis.

The osteoinductive potential of GDF-5 has been found smaller than that of other members of the BMP family, though numerous studies have confirmed its crucial role in skeletal morphogenesis. Several *in vitro* experiments have demonstrated that rhGDF-5 stimulates osteogenic differentiation and promotes angiogenic activity by increasing vascular endothelial growth factor gene expression in fat- or bone-marrow-derived stromal cells. The osteoinductive activity of rhGDF-5 has also been examined in numerous *in vivo* model systems [13].

Another GF extensively investigated for clinical applications is the platelet-derived growth factor (PDGF), which is synthesized by platelets, monocytes, macrophages, endothelial cells and osteoblasts. This is a dimeric molecule consisting of disulfide-bonded, structurally similar A- and B-polypeptide chains that combine to form homo- and heterodimers. The biologically most potent of these PDGFs is PDGF-BB, which has been thoroughly investigated. The PDGF isoforms exert their cellular effects by binding to and activating two structurally related protein tyrosine kinase receptors, called the alpha-receptor and the beta-receptor [16,17].

PDGF is stored in the alpha granules of circulating platelets and is released during blood clotting in the event of soft or hard tissue injury. Once it has been released from the platelets, PDGF binds to specific cell surface receptors and promotes rapid cell migration (chemotaxis)

and proliferation (mitogenesis) at the site of injury. In particular, *in vitro* and *in vivo* studies have demonstrated that PDGF is a potent chemotactic and mitogenic factor for gingival and periodontal ligament fibroblasts, cementoblasts and osteoblasts [18].

Since the first animal study conducted by Lynch and co-workers [19], extensive *in vitro,* preclinical and clinical studies have been performed using PDGF, alone or in combination with other GFs, for incrementing bone vertically and horizontally, and for treating periodontal and peri-implant defects. The positive outcomes of these studies provide strong evidence of the safety and predictably of rhPDGF combined with specific scaffolds in periodontal and peri-implant regeneration, suggesting promising clinical applications [18,20,21].

Although a large body of preclinical and clinical data has been obtained for only a few GFs, others have nonetheless been assessed for possible applications in clinical practice.

The activity and osteoinductive potential of fibroblast growth factor (FGF) have been the object of various studies [22-24]. FGF signaling reportedly interacts with BMP signaling in bone formation, showing a synergic action on osteogenesis [11].

Few studies have considered the use of parathyroid hormone (PTH) as a factor for modulating bone augmentation and healing [25]. PTH binding activates PTH1R to stimulate several downstream effectors and also drives the internalization of the PTH1R(PTH type I receptor)-TGFβRII (TGF-β type II receptor) complex, which attenuates both TGF-β and PTH signaling on bone development. The transcriptional factor/cAMP response element binding protein (CREB) mediates PTH signaling in osteoblasts, and the PTH-CREB signaling pathway serves as an effective activator of BMP-2 expression [11].

Transforming growth factor-β (TGF-β) [26-27], vascular endothelial growth factor (VEGF) [24], and insulin-like growth factor (IGF) [28] are also the object of studies regarding the biological properties of these bioactive molecules.

## 3. Clinical application of GFs in dentistry

Given the biological properties of GFs, a major focus of research has concerned the clinical application of the osteoinductive proteins, such as some BMPs, for enhancing new bone formation. Bone loss involving the teeth may be secondary to diseases such as periodontitis, cystic diseases or tumors, or the consequence of trauma. Alveolar bone augmentation procedures are often needed for the purpose of inserting dental implants for prosthetic rehabilitation.

Missing teeth can be replaced with prostheses supported on dental implants, which can only be inserted in patients with an adequate alveolar ridge height and/or thickness, so bone augmentation procedures enable implant treatments in cases in which it would otherwise not be an option. Bone augmentation procedures can be performed prior to implant placement (in a two-stage procedure), or during the same surgical procedure (one-stage procedure), using numerous materials and techniques.

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Various options have been described [29], including: autogenous bone grafts, allografts, xenografts, alloplastic grafts, barrier membranes for guided bone regeneration (GBR), growth factors (and BMPs in particular), platelet-rich plasma (PRP), inlay grafting, onlay grafting, ridge expansion, and distraction osteogenesis.

Tonetti et al. [30] described various techniques that have been developed to correct inadequate vertical and horizontal bone volumes, such as guided bone regeneration (GBR), sinus lift and onlay bone grafting.

Bone augmentation techniques have also been promoted as a means for treating periodontal and peri-implant diseases in an effort to regenerate lost periodontal or peri-implant soft and hard tissues [31-32].

Autogenous bone grafts are still considered the gold standard for bone repair in most cases, though there are some restrictions in their use in clinical practice because of the morbidity of the harvesting procedures and the limited amount of bone available. Many authors have consequently been studying the biocompatibility and effectiveness of other materials as potential substitutes for autogenous bone grafts.

The most recent and promising approach consists in applying osteoinductive growth factors to promote new bone formation (protein therapy) [33], providing a new alternative to autogenous grafts and other bone substitutes.

Combining growth factors with osteoinductive scaffolds may facilitate a faster and more significant enhancement of new bone formation thanks to the delivery of the growth factors at the site of the graft, and because their three-dimensional stability provides protection during the gradual replacement of the graft with newly-formed bone. Numerous materials have been used in combination with GFs, including inorganic bovine bone, porous hydroxyapatite and demineralized human bone matrix.

Numerous pre-clinical and clinical studies have looked into how GF implantation influences bone augmentation and implant osteointegration, focusing particularly on recombinant human BMP-2 (rhBMP-2), rhBMP-7 and recombinant human growth and differentiation factor-5 (rhGDF-5), combined with a variety of biomaterials used as scaffolds and delivery systems.

Although the potential value of GFs in alveolar bone regeneration and augmentation has been highlighted by numerous authors [6,31,34-35], it is still difficult to assess the different biological potential of each growth factor, because few analyses have compared different growth factors under identical *in vivo* conditions [24].

There is still much to learn about osteogenic growth factors: only a handful of growth and differentiation factors have been the object of clinical evaluation [6,18,25] and further studies are needed to identify predictable clinical outcomes.

### 3.1. Pre-prosthetic surgery for the purpose of dental rehabilitation with implants

Several surgical techniques and materials - including the use of GFs - have been introduced with a view to increasing bone volume in order to enable the placement of dental implants.

The systematic literature review conducted by Jung and coworkers [25] assessed the clinical, histological and radiographic outcomes after BMP-2, BMP-7, GDF-5, PDGF, and PTH had been used for localized alveolar ridge augmentation. Altogether, 74 studies met the authors' inclusion criteria, including 6 on the outcome of BMP-2 for localized alveolar ridge augmentation in humans; the remainder were pre-clinical studies involving BMP-2, BMP-7, GDF-5, PDGF, and PTH. For all the GFs other than BMP-2, no human studies met the inclusion criteria. Concerning the animal studies, most of those on BMP-2 (43 out of 45) showed a positive effect of this growth factor. Six of 8 studies reported a positive effect of BMP-7. The one animal study on GDF-5 spoke of a statistically significant increase in bone volume. Five of 10 studies involving the use of PDGF also reported a statistically significant increase in bone volume. Four animal studies identified a significantly greater bone regeneration in cases treated with PTH than in controls. In the six human studies, BMP-2 influenced local bone augmentation, with a dose-dependent increase in bone volume. The dose of BMP-2 delivered seemed to have an impact on treatment outcome, local bone regeneration being greater for higher BMP-2 doses [36-38], with a smaller decrease in bone height at extraction socket sites [39]. Four of these six human studies were designed as randomized-controlled clinical trials (RCT) [37-40], the other two as prospective cohort studies [36,41]. The locally-applied dose of BMP-2 ranged from 0.5 to 1.75 mg/ml, or 0.12 to 3.4 mg/patient, respectively. An absorbable collagen sponge (ACS) was used in five studies, while Jung et al. [40] used a demineralized bovine bone matrix (DBBM) as a carrier. The treatments included sinus floor augmentation [38,41], extraction socket preservation [36-37,39], augmentation of localized ridge defects [36], and lateral ridge augmentation combined with simultaneous implant placement [40].

The 16-week open-label study conducted by Boyne and coworkers [41] assessed the safety and efficacy of implanting BMP-2 delivered on an absorbable collagen sponge (rhBMP-2/ACS) for two-stage maxillary floor sinus augmentation. The dose of rhBMP-2 ranged from 1.77 to 3.40 mg per patient. Significant bone growth was documented by computed tomographic (CT) scans in all evaluable patients (11/12), with an overall mean response of 8.51 mm in height (±4.13 mm). Histology on core bone biopsies obtained when the dental implant was inserted confirmed the good quality of the bone induced by rhBMP-2/ACS.

In a more recent RCT, Boyne and colleagues [38] found no statistically significant differences in terms of the increase in ridge height, as measured using CT scans, between their treatment and control (bone graft) groups, and even a narrower ridge width in the former after using BMP-2/ACS in two-stage maxillary floor sinus augmentations.

Bianchi et al. [37] investigated the efficacy of different concentrations of rhBMP-2 in regenerating bone in alveolar defects in the anterior maxilla, reporting a positive outcome in terms of bone volume augmentation.

Another RCT [39] compared the efficacy of rhBMP-2 in two different concentrations, delivered on ACS, with placebo ACS alone in 80 patients requiring local alveolar ridge augmentation for buccal wall defects (> or =50% buccal bone loss around the extraction socket) immediately after tooth extraction of the maxillary bicuspids. They found no statistically significant effects of BMP-2 on the treatment outcome when a lower dose was used, but a statistically significant

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positive effect of a higher dose (1.50 mg/ml rhBMP-2/ACS). In addition, bone density and histology revealed no differences between newly-induced and native bone.

Finally, Jung et al. [40] tested whether adding rhBMP-2 to a xenogenic bone substitute mineral could improve guided bone regeneration in the case of bone defects requiring lateral bone augmentation procedures and simultaneous implant placement. Following implant insertion (baseline), the peri-implant bone defect height was measured from the implant shoulder to the first implant-bone contact. The authors reported a positive, but statistically insignificant effect of BMP-2 on the amount of newly-formed bone (37±11.2%) compared with the control group (30± 8.9%). On the other hand, they found more mature lamellar bone (76±14.4% versus 56±18.3%) and a greater area of bone-to-graft contact (57±16.2% versus 30±22.6%) at the BMP-2-treated sites.

Various methods have been described for increasing bone volume before or at the time of positioning implants [25], one of the best-documented of these methods being GBR for intra-oral bone augmentation. To overcome some of the drawbacks of this technique, e.g. a long treatment time, the difficulty of predicting any vertical bone augmentation, the risk of infection after membrane exposure, research has concentrated on the use of bioactive molecules that induce local bone formation. Using the GBR technique, the width and height of the alveolar ridge is increased in areas of insufficient bone volume by applying barrier membranes, alone or in combination with bone grafts or substitutes.

Misch [42] published a human case series of atrophic posterior mandible augmentation prior to implant insertion, using recombinant human BMP-2 2/absorbable collagen sponge (rhBMP-2/ACS) and titanium mesh. All the 10 implants involved in the study, inserted after a 6-month healing period, became integrated and were restored with single crowns.

Many *in vivo* studies used critical-size supra-alveolar peri-implant defect models and other bone augmentation methods simultaneously with implant insertion. In an animal study, Sigurdsson et al. [43] found that defect sites implanted with rhBMP-2⁄ACS showed signs of a statistically significant and clinically relevant vertical alveolar bone augmentation by comparison with controls (ACS). Although the titanium implant was osseointegrated after a 16-week healing interval, the BIC (bone-to-implant contact) was lower than in resident bone, as was to be expected; the newly-induced bone was often in a thin layer on the implant surface, probably due to the unpredictability of ACS in providing adequate space for new bone formation.

Wikesjö and colleagues [44] subsequently used a critical-size supra-alveolar peri-implant defect model to study the efficacy of an ePTFE GBR device in supporting rhBMP-2⁄-induced bone formation in dogs. The space-providing macro-porous membrane was characterized by the ability to prevent the compression of the rhBMP-2/ACS construct, while allowing for vascularization via the gingival connective tissue. The authors compared GBR alone with rhBMP-2(0.4 mg)⁄ACS and rhBMP-2(0.4 mg)⁄ACS combined with GBR. Histometric analysis on block biopsies after an 8-week healing interval revealed the best results in the third sample, i.e. the GBR-rhBMP-2⁄ACS combination, which revealed bone formation filling the dome-shaped GBR device, with a vertical bone gain at the turned implants averaging 4.7 ± 0.2 mm,

and an induced bone area of 9.6 ± 0.7 $mm^2$, generating a highly-significant correlation between the induced bone area and the space provided by the GBR device. This study highlighted the crucial importance of providing space in order to obtain clinically significant benefits from a BMP construct.

Jung et al. [45] ran a randomized-controlled clinical trial with a split-mouth design, in which implants were placed in sites exhibiting lateral bone defects and patients were randomly selected for treatment with demineralized bovine bone mineral and bioresorbable collagen membrane, with (test) or without (control) the addition of rhBMP-2. After an average healing period of 6 months, a reentry operation was performed for abutment connection and prosthetic reconstruction. At the 3-year follow-up, all 34 implants in all 11 patients were clinically stable and radiologically osseointegrated. At the 5-year follow-up, 32 implants were stable and functioning, while 2 were not re-examined because the patient had moved away. The survival rate of the implants examined at 3 and 5 years was therefore 100% for both the test and the control sites. The periapical radiographs of the test and control sites also showed no peri-implant radiolucency at the 3- and 5-year follow-up examination, demonstrating healthy peri-implant tissues with minimal marginal bone loss, and only minor prosthetic complications were recorded. In short, both the test and the control sites revealed excellent clinical and radiological outcomes after 3 and 5 years, with no statistically significant differences in any of the parameters examined (though the authors emphasized the need for a larger group of patients in future studies).

In a micro-CT study in dogs, Al-Hazmi and co-workers [20] assessed the efficacy of using PDGF-BB and xenografts, with or without collagen membranes, for GBR around immediate implants with buccal dehiscence defects. They concluded that using PDGF and xenografts resulted in greater BBT (buccal bone thickness), BBV (buccal bone volume), VBH (vertical bone height) and BIC (bone-to-implant contact) when used alone rather than in combination with a collagen membrane. Their results are consistent with the report from Simion et al. [46], who said that barrier membranes may interfere with the chemotactic effect of GFs on periosteal pluripotential mesenchymal cells.

Further studies are nonetheless warranted to investigate the influence of barrier membranes on the periosteal pluripotential mesenchymal cells [20].

Most of the clinical studies on rhPDGF have focused on periodontal and peri-implant regeneration, and only a few human studies have investigated ridge preservation for implant placement in extraction socket defects [47], or three-dimensional ridge augmentation [48].

In a pilot study, Nevins et al. [47] tested whether mineralized collagen bone substitute (MCBS) combined with recombinant human platelet-derived growth factor-BB (0.3 mg/mL) could generate enough viable bone in buccal wall extraction defects to enable implant placement.

In a more recent clinical study, Nevins and colleagues [49] focused on human buccal plate extraction socket regeneration with recombinant human platelet-derived growth factor BB or enamel matrix derivative. Buccal plate resorption is a critical issue when it comes to implant placement. They compared four groups: A (mineral collagen bone substitute [MCBS] scaffold alone), B (MCBS with recombinant human platelet-derived growth factor BB [rhPDGF-BB; 0.3

mg/mL]), C (MCBS with enamel matrix derivative [EMD]), and D (a combination of EMD with bone ceramic). Grafting was done at the time of extraction, advancing the buccal flap for primary closure. Histology on trephine core biopsies of the implant site performed 5 months later, at the time of implant placement, identified new bone healing around the biomaterial scaffolds with no statistically significant differences between the four treatment groups. There was a histomorphometric trend towards a greater quantity of new bone in the rhPDGF-BB-treated group, with the most favorable ridge morphology for the purposes of an optimal implant placement at reentry surgery.

Simion et al. [48] reported on two human cases of patients who underwent three-dimensional ridge augmentation using a xenograft combined with rhPDGF-BB. In the first patient, a deproteinized bovine block infused with rh-PDGF was attached to the alveolar crest with two screws to obtain a horizontal ridge augmentation. The second patient underwent a vertical ridge augmentation procedure involving deproteinized bovine bone particles embedded in a collagen matrix soaked in rhPDGF-BB. Three titanium dental implants were placed in each patient 5 months later with excellent clinical and histological outcomes, mean that rhPDGF-BB in combination with a deproteinized bovine graft has promise in applications for regenerating large three-dimensional alveolar defects in humans.

### 3.2. Dental implant surface coatings with GFs

Another interesting approach to enhancing alveolar ridge augmentation with a view to dental implant placement involves using implants coated with GFs.

Wikesjo and colleagues [35] reviewed the literature on implants coated with a bone-inductive factor capable of stimulating local bone formation and osseointegration. They concluded that rhBMP-2 can be delivered successfully for the purposes of inducing local bone formation and osseointegration by using screw-type endosseous oral implants with titanium oxide surfaces with open pores as a carrier. They also found that purpose-designed implant surfaces coated with rhBMP-2 resulted in the formation of Type II bone and significant osseointegration without any need for biomaterials or devices for GBR.

In an *in vivo* animal model, Susin et al. [50] used the critical-size supra-alveolar peri-implant defect model to assess the potential of a purpose-designed porous titanium oxide implant surface coated with rhBMP-7 for inducing alveolar bone formation and enhancing osseointegration. The animals received implants coated with rhBMP-7 at 1.5 or 3.0 mg/ml randomized to the contralateral jaw quadrants. The authors found clinically relevant bone formation and osseointegration with no statistically significant differences in terms of bone formation between the sites treated with rhBMP-7 at 1.5 or 3.0 mg/ml. Histology showed an increase in the height and area of the bone, and the newly-formed bone exhibited the same characteristics as the contiguous resident bone. Their observations support the significant clinical value of rhBMP-7 in inducing bone regeneration, but the authors made the point that higher concentrations were associated with some local side effects.

Other authors [e.g. 51-52] have investigated *in vivo* the potential of an rhGDF-5 coating on an oral implant with a porous titanium oxide surface for stimulating local bone formation, including osseointegration and vertical augmentation of the alveolar ridge.

Polimeni and co-workers [51] examined a bilateral critical-size, 5 mm, supra-alveolar peri-implant defect model in dogs. Six animals received implants coated with 30 or 60 μg rhGDF-5, and another six animals received implants coated with 120 μg rhGDF-5 or left uncoated (controls). The implants coated with rhGDF-5 displayed only limited peri-implant bone remodeling in the resident bone, as measured using fluorescent bone markers, with the 120 μg dose coinciding with a more advanced remodeling than the 60 and 30 μg doses. These results suggest a dose-dependent osteoinductive and/or osteoconductive effect of rhGDF-5-coated oral implants. Leknes et al. [52] performed an *in vivo* study in dogs that consisted in placing different kinds of implant in the alveolar ridge of the posterior mandible following the surgical extraction of the premolars and reduction of the alveolar ridge. Six animals were treated with implants coated with rhGDF-5 in doses of 30 or 60 μg/implant in contralateral jaw quadrants, while six received implants coated with rhGDF-5 at 120 μg/implant or uncoated implants (for control purposes), using a split-mouth design. The radiographs showed a dose-dependent formation of mineralized tissue significantly greater than around the uncoated implants, the greatest increase corresponding to the implants coated with 60 μg and 120 μg of rhGDF-5, and amounting to approximately 2.2 mm in both cases at 8 weeks. The authors also reported no adverse events, such as peri-implant bone remodeling, implant displacement, or seroma formation.

The above-mentioned studies indicate that these GFs have great potential for stimulating clinically relevant local bone formation, though it should be emphasized that further studies are essential to address their most appropriate dosage, carriers, and applications, as well as the long-term prognosis of GF-coated titanium implants.

### 3.3. Maxillary sinus lift procedure

Sinus floor elevation with immediate or delayed dental implant placement is a well-known technique for dental rehabilitation in cases of severe atrophy of the posterior maxilla due to the extension and pneumatization of the maxillary sinus. Many materials, such as autografts, xenografts, and synthetic bone substitutes, have been shown to achieve acceptable clinical results when used in maxillary sinus floor augmentations [53]. The use of GFs with various carriers and dosages has recently been investigated in combination with sinus augmentation procedures too.

Ho and colleagues [54] assessed the efficacy of various bioimplants used in maxillary sinus lift procedures with the lateral window approach in a rabbit model. They compared particulated autogenous bone, demineralized bone matrix (DBM), DBM combined with purified BMP-7 (BMP-7/DBM bioimplants), and bioimplants consisting of a poloxamer gel with BMP-7 in two different doses. In their animal model, BMP-containing bioimplants had produced more new bone and a greater new bone surface area at 2 weeks than autografts, but the advantage of these bioimplants subsequently seemed to be lost, since the differences between the bioimplants and the autografts had disappeared by 8 weeks. The authors concluded that BMP-

containing bioimplants prompt a more rapid bone formation, possibly offering a greater implant stability earlier in the healing period, and therefore enabling clinicians to place osseointegrated implants in augmented maxillae sooner after grafting.

In a clinical study, Boyne and colleagues [38] compared different concentrations of rhBMP-2 (0.75 and 1.5 mg/mL), delivered on an absorbable collagen sponge (ACS) carrier, with bone grafts to identify a safe and effective concentration of rhBMP-2 for use in maxillary sinus floor augmentation procedures. Judging from density measurements on CT scans obtained before and 4 months after treatment, and 6 months after functional loading of the dental implants, and from core biopsies obtained at the time of placing the dental implant, they established that the 1.5 mg/mL dose of rhBMP-2/ACS was more appropriate in a pivotal, randomized, multicenter study to compare rhBMP-2/ACS with conventional bone graft for staged maxillary sinus floor augmentation to support dental implants for long-term functional loading.

These data prompted a randomized, parallel evaluation of rhBMP-2/ACS and autogenous bone grafts for two-stage maxillary sinus floor procedures [55]: 160 individuals with less than 6 mm of native bone height in the posterior maxilla were randomized for treatment with 1.5 mg/mL rhBMP-2/ACS or an autograft. Height and density measurements were obtained on CT scans, and core biopsies obtained at the time of dental implant placement underwent histological examination. A significant amount of new bone had formed by 6 months postoperatively in both treatment groups, but there was a significant difference in the density of the newly-induced bone at the 6-month follow-up, which was denser in the bone graft group than in the group treated with rhBMP-2/ACS. Six months after dental restoration (functional loading), however, the bone induced in the rhBMP-2/ACS group was significantly denser than in the bone graft group. No major differences emerged between the two groups in terms of the histological parameters. 17% of the patients in the autograft group experienced long-term parasthesia, pain, or gait disturbance relating to the bone graft harvest. Adverse reactions frequently recorded in the rhBMP-2/ACS group related to excessive facial swelling, and this edema was attributed to the chemotactic cellular recruitment to the site of rhBMP-2 implantation and neovascularization of the grafted area; although it was severe, this edema did not adversely affect the outcome. This study confirmed the efficacy and safety of rhBMP-2/ACS by comparison with bone grafting for sinus floor augmentation, given the morbidity, cost, and increased surgical time associated with the harvesting of autogenous bone.

Kao and coworkers [56] measured the bone formation after a lateral window sinus augmentation with recombinant human BMP-2/ absorbable collagen sponge (rhBMP-2/ACS) in combination with Bio-Oss by comparison with the results achieved with a Bio-Oss graft alone. Histology demonstrated that less new bone formed in patients treated with rhBMP-2/ACS + Bio-Oss than in those treated with Bio-Oss alone, pointing to a negative effect on bone formation of combining rhBMP-2 with Bio-Oss for maxillary sinus augmentation.

Gruber and coworkers [57] studied a GF closely related to the BMP family - the recombinant human growth and differentiation factor-5 (rhGDF-5) - in an *in vivo* study involving the use of different materials in sinus floor augmentation procedures in Goettingen miniature pigs. They demonstrated that associating rhGDF-5 with β-tricalcium phosphate

(β-TCP) enhanced bone formation by comparison with the results obtained using the β-TCP carrier material alone.

In a further study using a split-mouth study design, the same authors [13] compared rhGDF-5-coated β-TCP with particulated autogenous bone grafts combined with the scaffold material (β-TCP). In each minipig, the sinus floors were augmented (simultaneously inserting the dental implants) with β-TCP mixed with autogenous cortical bone chips on one side, and using β-TCP coated with two different concentrations of rhGDF-5 on the contralateral side. Histology and histomorphometric analyses demonstrated that rhGDF-5-coated β-TCP not only enhanced new bone formation, but also - by comparison with a combination of β-TCP and autogenous bone chips - induced a significant increase in VD (volume density) and BIC (bone-to-implant contact) in the augmentation material.

Stavropoulos et al. [58] ran a prospective, multicenter, randomized clinical trial to examine the histological outcome of maxillary sinus lifting with rhGDF-5/β-TCP or β-TCP and autogenous bone (β-TCP/AB) composite. Thirty-one patients requiring unilateral maxillary sinus floor augmentation with a residual alveolar bone height <5 mm were treated using a lateral window approach. Cylindrical biopsies were harvested with a trephine bur during implant site preparation 3 or 4 months after sinus floor augmentation (three groups (a) rhGDF-5/b-TCP and a 3-month healing period, (b) rhGDF-5/b-TCP and a 4- month healing period, and (c) b-TCP/AB and a 4-month healing period). Histological and histometric analyses showed that sinus augmentation with rhGDF-5/β-TCP resulted in new bone in comparable amounts and of similar quality to the bone obtained with a β-TCP/AB composite graft, suggesting that rhGDF-5/β-TCP could eliminate the need for AB grafting in sinus lift procedures.

Though these favorable regenerative findings are encouraging, further studies are needed to ascertain the influence of GFs on the amount and quality of new bone formation, and on the implant survival rate after sinus lift procedures.

### 3.4. Periodontal regeneration

Periodontitis is a widely prevalent inflammatory disease of the tissues supporting the teeth, characterized by a progressive loss of bone and attachment.

The ultimate goal of periodontal therapy is the regeneration of periodontal tissues, which consists in stimulating new cementum formation, new alveolar bone apposition, and a functionally-oriented periodontal ligament reconstruction. Various techniques have been suggested for promoting periodontal tissue regeneration, using different bone graft materials that have gained clinical acceptance in the treatment of periodontal defects.

To overcome the weaknesses of conventional regenerative procedures, the predictability of which may be limited to selected case types, using GFs with biocompatible scaffolds to promote tissue regeneration may represent a new and promising periodontological approach.

After preliminary *in vitro* experiments, extensive *in vivo* preclinical studies have been performed to assess the potential and safety of using various GFs, alone or in combination, to treat periodontal defects.

A recent animal study by Oortgiesen et al. [23] investigated the regenerative potential of an injectable macroporous calcium phosphate cement (CaP) combined with BMP-2 or fibroblast growth factor-2 (FGF-2) in intrabony defects. After 12 weeks, only the CaP revealed limited effects on both periodontal ligament (PDL) and bone healing, while a good response in terms of bone healing was also seen with CaP/BMP-2 and CaP/FGF-2. The best PDL healing scores coincided with the combined CaP/FGF-2 treatment, suggesting that associating a topical application of FGF-2 with an injectable CaP might be a promising treatment for the purposes of periodontal regeneration.

Ishii and colleagues [22] investigated the effect of the combined use of basic FGF-2 and beta tricalcium phosphate (β-TCP) on root coverage in a dog model, finding that FGF-2/β-TCP enhanced the formation of new bone and cementum without any significant root resorption.

Kitamura et al. [59] undertook a multi-center, randomized, double-blind, placebo-controlled, dose-finding study on the potential of local applications of FGF-2 in periodontal regeneration. Modified Widman periodontal surgery was performed, during which 200 μL of the investigational formulation containing 0% (vehicle alone), 0.2%, 0.3%, or 0.4% FGF-2 was administered to 2- or 3-walled vertical bone defects in 253 adult patients with periodontitis. The primary outcome was the percentage of bone fill visible on radiographs 36 weeks after administering the treatment. All the doses of FGF-2 were significantly superior to the vehicle alone ($p < 0.01$) in terms of the percentage of bone fill, and this percentage peaked in the 0.3% FGF-2 group. No significant differences were observed between the four groups in terms of the regained clinical attachment (CAL), with all patients scoring around 2 mm (this was judged to be due to the different healing patterns between the FGF-2 groups and the 'vehicle alone' group). Conventional periodontal surgery (which corresponds to the 'vehicle alone' group) usually gives rise to long junctional epithelial attachments, but manual probing cannot precisely distinguish fibrous from epithelial attachments, so the difference in healing pattern cannot be reflected in the CAL regained by the different treatment groups. This limitation could have been overcome by histology, but this was not done for ethical reasons. No clinical safety issues emerged in this study. These results support the efficacy and safety of topical FGF-2 applications for periodontal regeneration in humans.

When implanted in furcation defects exposed surgically or by inflammatory processes in *Papio ursinus*, recombinant human osteogenic protein-1 (hOP-1) or BMP-7 tends to induce cementogenesis with the insertion of *de novo* generated Sharpey's fibers. Long-term studies on *P. ursinus* after hOP-1 implantation show a highly-organized periodontal ligament space with periodontal ligament fibers cursing from the newly-formed and mineralized cementum to the regenerated alveolar bone, with a multitude of supporting capillaries throughout the periodontal ligament space [60].

In an experimental study by Teare et al. [27], binary applications of hOP-1 and hTGF-β(3) were implanted in Class II furcation defects of the mandibular molars of Chacma baboons (*P. ursinus*) to induce periodontal tissue regeneration. Sixty days after implantation, the animals were killed and histological and histomorphometric studies led the authors to conclude that

hOP-1 and hTGF-β(3) in Matrigel(®) matrix induced substantial periodontal tissue regeneration and cementogenesis.

In their review, Ripamonti et al. [61] emphasized the induction of bone formation by the osteogenic proteins of the TGF-beta superfamily in the nonhuman primate, *P. ursinus*.

In a recent study in beagle dogs, Kim and co-workers [62] compared a candidate β-tricalcium phosphate (β-TCP) carrier technology with the absorbable collagen sponge (ACS) benchmark for supporting rhGDF-5-stimulated periodontal wound healing/regeneration in intrabony periodontal defects. Both solutions stimulated the formation of functionally-oriented periodontal ligament, cellular mixed-fiber cementum, and woven/lamellar bone, but bone regeneration (height and area) was significantly greater for the rhGDF-5/β-TCP construct. The structural integrity of the β-TCP carrier preventing compression while providing a framework for bone ingrowth may account for these results.

A phase IIa randomized controlled clinical and histological pilot study was conducted to assess rhGDF-5/β-TCP for periodontal regeneration [63]. Twenty chronic periodontitis patients participated in the study, each with at least one tooth scheduled for extraction with a probing depth (PD) ≥6 mm and an associated intrabony defect ≥4 mm following basic periodontal therapy. Participants (one defect/patient) were randomized to receive open flap debridement (OFD) + rhGDF-5/β-TCP (n = 10) or OFD alone (control; n = 10). Both protocols resulted in statistically significant clinical improvements. Descriptive statistics showed a greater reduction in PD after OFD with rhGDF-5/β-TCP than after OFD alone (3.7 ± 1.2 versus 3.1 ± 1.8 mm; $p = 0.26$), as well as less gingival recession (0.5 ± 0.8 versus 1.4 ± 1.0 mm; $p < 0.05$) and a greater CAL gain (3.2 ± 1.7 versus 1.7 ± 2.2 mm; $p = 0.14$) at the deepest aspect of the defect. Block biopsies of the defect sites were collected 6 months after surgery and prepared for histology. Five biopsies (1 rhGDF-5/β-TCP; 4 OFD) were deemed unsuitable for histological or histometric evaluation. Bone regeneration height (2.19 ± 1.59 versus 0.81 ± 1.02 mm; $p = 0.08$) and PDL (2.16 ± 1.43 versus 1.23 ± 1.07 mm; $p = 0.26$), cementum (2.16 ± 1.43 versus 1.23 ± 1.07 mm; $p = 0.26$) and bone regeneration area (0.74 ± 0.69 versus 0.32 ± 0.47 $mm^2$; $p = 0.14$) were greater at sites treated with rhGDF-5/β-TCP compared to controls. These differences failed to reach statistical significance, however, and the authors said that further studies on larger samples will be needed to verify these findings.

The potential of PDGFs for promoting new bone formation and/or periodontal wound healing/regeneration has been examined in a variety of pre-clinical animal models. *In vivo* experimental studies have been performed using PDGF-BB alone or in combination with other GFs, such as insulin-like growth factor (IGF), and shown that these growth factors promoted new bone, cementum and periodontal ligament formation *in vivo*.

The first human clinical trial testing the effect of rhPDGF/rhIGF-I in periodontal defects was reported by Howell and colleagues [64] with promising results.

Early human clinical studies used rhPDGF-BB combined with bone allografts. An alternative is to use a synthetic system, such as β-tricalcium phosphate (β-TCP). Since rhPDGF applications have proved clinically effective in the treatment of intrabony defects, this growth factor has also been considered for the treatment of soft tissue recession defects [18].

Jayakumar and coworkers [65] ran a double-blind, prospective, parallel, active-controlled, randomized, multi-center clinical trial on the efficacy and safety of rhPDGF-BB with β-TCP in human intraosseous periodontal defects. Fifty-four patients with periodontal osseous defects were randomly grouped for treatment with rhPDGF-BB/β-TCP or β-TCP alone. A total number of 50 defects in 25 patients in the rhPDGF-BB/β-TCP group and 25 in the β-TCP group were ultimately available for statistical analysis. The radiographic parameters considered were linear bone growth (LBG) 6 months after surgery and percent bone fill (% BF), both of which were found significantly higher in the rhPDGF-BB/β-TCP group than in the β-TCP group. There also emerged a significantly higher area under the curve for clinical attachment level gain from 0 to 6 months, and a greater reduction in PD at the third and sixth month than after β-TCP treatment alone. The implantation of rhPDGF-BB/β-TCP for the treatment of intraosseous periodontal defects was safe and well tolerated, and resulted in clinically and statistically significant improvements in bone formation parameters and soft tissue outcomes.

Preliminary investigations thus indicate that GFs have great potential for improving periodontal regeneration, but randomized clinical trials must be conducted to gain a better understanding of the role of GFs in periodontal treatments, focusing particularly on establishing the safety and efficacy of their application.

## 4. Growth factor delivery systems

The great potential of GFs in bone regeneration has been discussed by numerous authors [6,31,34-35]. BMP-2 and BMP-7 have a marked effect on bone and cartilage growth and the maintenance of homeostasis during bone remodeling [66]. One of their limitations, on the other hand, seems to be the unpredictable nature of the resulting tissue regeneration *in vivo*. It has been suggested that the clinical efficacy of recombinant human forms of BMPs (rh-BMPs) depends on the carrier system used to ensure an effective delivery of adequate protein concentrations to the site being treated [67]. BMPs are soluble proteins and, delivered in a buffer solution, they undergo rapid degradation, leading to an insufficient bioavailability. Other factors, such as protein competition, enzymatic activity, temperature, pH and salt concentration, may also influence the total amount of active protein available immediately after its administration [68].

In 2007 Giannoudis et al. [69] came up with the "Diamond Concept" to describe the conditions needed for osteogeneration, i.e. mechanical stability at the site of the defect, and osteogenic cells combined with osteoinductive growth factors and a suitable carrier or delivery system.

The main purpose of the delivery system is to ensure adequate protein concentrations at the defect site for as long as it takes to enable the regenerative cells to migrate, proliferate and differentiate [33].

A localized, controlled release is also necessary to prevent any unwanted and uncontrolled ectopic bone formation in non-bony body tissues [70]. Supra-physiological concentrations resulting from imperfect GF release kinetics have been correlated with severe clinical

complications, including generalized hematomas in soft tissues and peri-implant bone resorption. Other potential concerns theoretically include carcinogenicity and teratogenic effects [70].

Few authors have investigated the influence of GF release kinetics on bone regeneration. In physiological bone repair, some growth factors (such as BMP-2) are expressed mainly during the early inflammatory phase. Others are up-regulated during the chondrogenic and osteogenic phases, and have a biphasic expression pattern or are constitutively expressed [33]

In vivo studies demonstrated that higher BMP-2 retention times were more osteoconductive [71], and that prolonged BMP-2 delivery enhanced the protein's osteogenic efficacy by comparison with a shorter-term delivery of an equivalent dose in a rat model [72]. Release should preferably be sustained over time, either in large single doses or in multiple smaller-dose applications. In evaluating the timing of the protein release, it is important to consider the dynamic nature of the healing zone, which depends on the type, location and appearance of the defect, the patients' age and gender, their hormone and nutritional state, and any diseases, as well as other parameters influencing release rate, including the protein's size and conformational changes, solubility, polymer/scaffold composition/geometry, and molecular weight [33].

Dose and concentration parameters are available for orthopedic clinical applications, where different anatomical sites require different therapeutic doses depending on the degree of vascularization, defect size and the number of resident responding cells. Supraphysiological dosages range from 0.01 mg/ml in small animal models (e.g. rats) to 0.4 mg/ml in rabbits, to more than 1.5 mg/ml in non-human primates [33].

Growth factor release from a delivery system may be diffusion-controlled, chemical or enzymatic reaction-controlled, solvent-controlled, or controlled by a combination of these mechanisms. Diffusion-controlled release is governed by the protein's solubility and diffusion coefficient in the aqueous medium, protein partitioning between the aqueous medium and the material of the delivery system, protein loading and the diffusional distance. Chemical or enzymatic reaction-controlled systems include erodible systems, in which the protein is physically immobilized in the carrier matrix and released as the carrier undergoes degradation and dissolves. In solvent-controlled systems, the protein is embedded in a carrier matrix and a diffusional release occurs as a consequence of the rate-controlled penetration of the solvent (water) in the system [33].

Several GF delivery systems and carriers have been suggested for use in bone regeneration applications in an effort to find the optimal strategy for optimizing their clinical effectiveness and minimizing complications.

Delivery systems and carriers used for bone GFs should meet general requirements (Table 1) such as biocompatibility, predictable biodegradability, and the ability to provoke appropriate inflammatory responses. They must also have the following features: easy and cost-effective to manufacture; stability; easy handling and storage [33].

| |
|---|
| Biocompatibility |
| Predictable biodegradability |
| Low immunogenicity and antigenicity |
| Enhancement of cellular vascularization and attachment |
| Affinity with BMPs and bone |
| Maintenance and enhancement of BMP bioactivity |
| Malleability and ease of manufacture |
| Safety, stability, sterility, availability and cost-effectiveness |
| Regulatory agency approval for the clinical application of interest |
| Controlled protein release at an effective dose for the appropriate period of time |

**Table 1.** General requirements for BMP delivery systems

Carrier materials have been generally divided into four classes (Table 2): natural-origin polymers (collagen, hyaluronic acid, gelatin hydrogel complex, alginates and chitosan); inorganic materials (synthetic bone grafts, hydroxyapatite, calcium phosphates and bioactive glasses); synthetic biodegradable polymers (polylactic acid PLA, polyglycolide PLG, and their polymers PLGA, cholesterol-bearing pullulan nanogel CHPA), and composites (combinations of materials from the above different classes) [33].

To date, only BMP-2 and BMP-7 have been approved by the US Food and Drug Administration for human use in specific orthopedic applications, delivered using absorbable collagen sponges [33].

### 4.1. Collagen

Collagen is the protein most abundant in the connective tissue of mammals and the main non-mineral component of bone. It has been prepared in powders, membranes, films and implantable absorbable sponges, as well as in aqueous forms. Although it is versatile and easy to manipulate, the manufacture of collagen carriers is highly sensitive to several factors (including mass, soaking time, protein concentration, sterilization, buffer composition, pH and ionic strength) that directly affect rhBMPs binding [73]. Absorbable collagen sponges (ACS) have been evaluated in numerous in vivo models and clinical trials [6, 38,74-76]. In patients requiring staged maxillary sinus floor augmentation, rhBMP-2/ACS safely induced adequate bone formation for the purpose of placing and functionally loading endosseous dental implants [38]. The use of rhBMP-2/ACS without any concomitant bone grafting materials in critical-size mandibular defects prompted an excellent regeneration in a case review of 14 patients [75]. On the other hand, a recent study by Kao et al. demonstrated a more limited bone formation after a lateral-window sinus augmentation procedure involving rhBMP-2/ACS combined with Bio-Oss than when Bio-Oss was used alone [56].

Although they do away with the need to harvest autologous bone (with the associated pain), the use of animal-derived collagens is limited by their xenogenic nature: anti-type I collagen antibodies reportedly developed in almost 20% of patients treated with rhBMP-2/ACS [6]. In addition, collagen sponges are usually sterilized with ethylene oxide prior to soaking the

| Class | Types | Advantages | Disadvantages |
|---|---|---|---|
| Natural polymers | Collagen (gels, nano fibers, scaffolds and films)<br>Fibrin glue<br>Alginate and chitosan | Biocompatible, biodegradable, soluble in physiological fluids, natural affinity with BMPs | Immunogenicity (xenogenic), pathogen transmission, sensitivity to sterilization process |
| Inorganic materials | Synthetic bone grafts<br>CPC (calcium phosphate cement)<br>Bioactive glasses, hydroxyapatite, hyaluronic acid, tricalcium phosphates, metal, ceramics and calcium sulfate | Osteoconductive, affinity with BMPs | Brittle, difficult mold, some formulations are exothermic |
| Synthetic polymers | PLLA and PGLA and their copolymers<br>CHPA | Easy to process and sterilize, flexible to tailor and reproducible, excellent chemical and mechanical properties | Inflammatory response, localized pH drop and limited biological function |
| Composites | Collagen-HA and titanium PLLA | Depending on the combination of the different materials' characteristics | Complex to manufacture |

**Table 2.** Major classes of carrier materials

sponge in the BMP solution, and this can affect the GF release kinetics or the protein's bioactivity [73].

### 4.2. Alginate and chitosan

Alginate is a non-immunogenic polysaccharide used in a wide range of tissue engineering applications for its gel-forming properties. Alginate hydrogels allowing for a controlled, prolonged release of BMPs have only been studied in the preclinical phase, with promising results in vitro [72,77]

Chitosan is a cationic glucopolymer well known for its biological, chelating and adsorbing properties, and has been used as a BMP-2 carrier in a rat critical-size mandibular defect model, with positive results on histological and histomorphometric analysis [78].

### 4.3. Hyaluronic acid

Hyaluronic acid is a naturally-occurring biopolymer that plays a significant part in wound healing. It has been associated with an improved bone formation in mandibular defects by comparison with collagen sponges, when both were used to carry rhBMP-2 [79].

### 4.4. Hydroxyapatite

Hydroxyapatite (HAP) is well known for its osteoconductivity and has been widely used as a bone substitute material in clinical practice since the 1970s because of its ability to bond directly with bone [80]. Synthetic HAP comes in ceramic or non-ceramic, cementable forms, and has been evaluated as a scaffold and a controlled-release carrier, demonstrating lack of resorption and limited bone induction [6]. It has been combined with tri-calcium phosphates, collagen and other materials to form rigid, resorbable, porous carriers, in which case delivery and bone formation were generally found better than when HAP was used alone [81,82].

### 4.5. Synthetic biodegradable polymers

Unlike natural polymers and collagen, synthetic polymers pose no problem of immunogenicity or risk of disease transmission.

The most commonly-used polymers are polylactic acid (PLLA) and polyglycolic acid (PLGA). Bioresorbable PLLA/PLGA copolymers have been found superior to collagen when used to deliver rh BMP-2 to mandibular defects in the rat [83].

### 4.6. Bone grafts and derived composite materials

Bone grafts act as scaffolds for the ingrowth of vessels and bone-forming cells. During this osteoconductive bone regeneration process, the scaffold allows for bone to grow on its surface and inside the pores in the material. Given the biological limitations of other osteoconductive materials and the donor site morbidity after bone harvesting, the combination of osteoconductive scaffolds with osteoinductive proteins, such as BMPs, has been a major focus of research. [13,84]

Bone substitutes for use in dental and maxillofacial surgery are classified in three groups according to their origin. Allogenic bone grafts are derived from human donors, xenogenic bone grafts from other species (mostly bovine, but also equine, porcine and coralline), and the last group comprises the synthetically-produced materials. Synthetic bone grafts aim to imitate the natural bone's structure. The most widely used are the calcium phosphates, including hydroxyapatite, tri-calcium phosphates (TCP) and composites of the two. By means of a thermal treatment (sintering) and subsequent cooling they can be transferred into ceramics with a very solid but porous structure and a rough surface closely resembling human bone.

Recent studies have reported successful bone regeneration after grafting on periodontal defects, using sinus floor elevation techniques, and in post-extraction socket defects using TCP carriers [58,65, 85].

Clinical studies reporting results of GFs delivery systems in oral surgery are revised in Table 3.

Some authors have also investigated the application of GFs to dental implant surfaces to stimulate local bone formation and osteointegration. In preclinical studies, functionalized titanium implant surfaces coated with rhBMP-2 have been shown to be able to stimulate bone formation around implants [35, 86]

| References | Study design | Total number of patients | Protein | Carrier | Application | Main findings |
|---|---|---|---|---|---|---|
| Jung et al. 2003 [40] | RCT | 11 | rhBMP-2 | Xenogenic bone (Bio-Oss) | Maxillary implant placement | rhBMP-2 has the potential to predictably improve and accelerate guided bone regeneration therapy |
| Boyne et al. 2005 [38] | RCT | 48 | rhBMP-2 | ACS | Maxillary sinus floor elevation | rhBMP-2/ACS safely induced adequate bone for the placement and functional loading of dental implants |
| Herford and Boyne 2008 [75] | Case review | 14 | rhBMP-2 | ACS | Mandibular defect | Bone formation could be identified radiographically after 5 to 6 months |
| Van den Bergh et al. 2000 [76] | | 3 | rhBMP-2 | Type I collagen | Maxillary sinus floor elevation | Potential for initiating bone formation in the human maxillary sinus within 6 months after a sinus floor elevation, but its behavior is currently not sufficiently predictable in this application |
| Kao et al., 2012 [56] | Clinical trial | | rhBMP-2 | ACS and xenogenic bone | Sinus floor elevation | Less bone formed in patients treated with the rhBMP-2/ACS/xenogenic bone device |
| Alonso et al. 2010 [89] | RCT | 16 | rhBMP-2 | Collagen | Alveolar defect closure in cleft lip and palate patients | Satisfactory bone healing at 6 months and reduced morbidity |
| Stavropoulos et al. 2011 [63] | RCT | 20 | rhGDF-5 | β-TCP | Regeneration of periodontal defects | Greater alveolar regeneration, differences not statistically significant |
| Nevins et al. 2011 [49] | Cohort study | | rhPDGF | Mineral collagen scaffold | Socket preservation | No statistically significant differences were observed |
| Stavropoulos et al. 2011 [58] | RCT | 31 | rhGDF-5 | TCP | Sinus floor elevation | Comparable amount and similar quality of bone formation as in controls |
| Jayakumar et al. 2011 [65] | RCT | 54 | rhPDGF | TCP | Regeneration of periodontal defects | Increased bone formation and soft tissue healing |

| References | Study design | Total number of patients | Protein | Carrier | Application | Main findings |
|---|---|---|---|---|---|---|
| McAllister et al. 2010 [85] | RCT | 12 | rhPDGF | β-TCP | Socket preservation | Similar histological findings at 3 months |
| Triplett et al. 2009 [55] | RCT | 160 | rhBMP-2 | ACS | Sinus floor elevation | Induced bone was significantly denser, no marked differences in histological parameters. |

**Table 3.** Clinical studies on GF delivery systems applicable in oral surgery

### 4.7. Gene delivery methods

The potential applications of gene therapy have recently expanded to include the local treatment of bone defects. Gene transfer methods may circumvent many of the weaknesses of protein delivery to soft tissue wounds. The application of growth factors or soluble forms of cytokine receptors by means of gene transfer offers a greater sustainability than the use of a single protein application. Gene therapy may make growth factors more readily bio-available.

Gene transfer is accomplished by using viral and non-viral vectors. Examples of viral vectors are retroviruses, adenoviruses (Ads), and adeno-associated viruses (AAV), and non-viral vectors include plasmids and DNA polymer complexes.

Some authors have studied gene delivery via adenoviral or liposomal vectors carrying information for encoding recombinant human GFs combined with a collagen matrix in animal models [87,88].

## 5. Conclusion

The role of growth factors for alveolar bone regeneration in dentistry is a recent field of research, with a relative paucity of clinical studies. Findings seem to demonstrate a positive effect of GFs on intraoral hard and soft tissues healing, and the bone regeneration associated with implant therapy represents one of the main scenarios of interest. For the time being, however, the application of GFs in this field is limited by the dubious results, complications and side effects encountered so far. In particular, one of the main problems seems to be the relationship between the GF delivery and the timing of the healing process. Among the delivery systems tested to date, only collagen matrices have correlated with successful clinical results, albeit with some limitations. Other potential delivery systems have been studied only in a few animal models, and the currently available data are not enough for any final conclusions to be drawn. The development of dedicated and more "sophisticated" GF delivery systems is probably the most interesting area of research for the future.

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