

Growth Factors and Craniofacial Surgery

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Abstract: The specialty of craniofacial surgery is broad and includes trauma, aesthetics, reconstruction of congenital deformities, and regeneration of tissues. Moreover, craniofacial surgery deals with a diverse range of tissues including both “soft” and “hard” tissues. Technological advances in materials and biological sciences and improved surgical techniques have remarkably improved clinical outcomes. The quest to raise the bar for patient care continues to inspire advances for predictable biological regeneration of soft and hard tissues. As a consequence of this quest for advancement, a wide spectrum of biologicals is becoming available to surgeons. Is the use of recombinant DNA engineered biologicals daring? Sensible? Logical? Timely? Safe? It is crucial for the practicing craniofacial surgeon to take a step back periodically and carefully review the biological factors that have the potential for dramatically altering the discipline of craniofacial surgery. With this emphasis, the coauthors of this article will focus on growth factor technology underscoring bone tissue regeneration. As the 21st-century matures, recombinant human biologicals will have an overwhelming impact on the practice of craniofacial surgery.

Key Words: Craniomaxillofacial surgery, growth factors, platelet-rich plasma, platelet-derived growth factor BB, bone morphogenetic protein 2

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Craniofacial surgery addresses numerous and varied challenges in the craniomaxillary-mandibular complex. Likewise, within this complex, there is a constellation of cell phenotypes and tissues. As a meaningful focus for this review, we emphasize a highly specialized tissue: bone.

Facial injuries secondary to trauma are one of the most common indications for craniofacial surgery. The objective of traumatic fracture treatment is to restore form and function. Facial fractures are commonly classified by severity and the mechanism of injury.¹ Congenital defects, such as cleft palates, require augmen-

tation of the bone and soft tissue to reconstruct the structures of the face and skull.² Periodontal disease, which affects the structures of the jaw, is one of the most prevalent diseases in the United States and western Europe, with more than two thirds of the elderly population being afflicted.³ Given the number of craniofacial applications, there is a need for consistent, predictable outcomes.

In past years, the field of craniofacial surgery has experienced advances that have significantly improved clinical outcomes. Advances have included improved surgical techniques, such as distraction osteogenesis and endoscopic procedures, as well as technological advances involving computer simulation, intraoperative navigation, and three-dimensional imaging.⁴ Moreover, innovation in biomaterials that are biocompatible and biomechanically matched bone has improved surgical success and reduced morbidity.

There is an abundance of surgical techniques and technologies aimed at bone augmentation and osteointegration, and the decision-making process over which technology to use may be complex, especially when scientific support is limited, and preclinical data are confined to in vitro studies and diverse, unique small animal studies.⁵ It is not unexpected, theretofore, that recombinant DNA-derived human growth factors for bone regeneration have provided a rich landscape for both clinical utility and surgical efficacy, but also controversy.

Growth Factors and Biologicals

The isolation and identification of bone morphogenetic proteins (BMPs),⁶ stemming from Marshall Urist's⁷ observations that demineralized bone matrix could induce bone formation, provided the framework for biologically derived growth and morphogenetic factors to enhance bone repair. Although biological materials, such as autograft, allograft, and demineralized bone matrix have a rich history in craniofacial surgery, recent technological advances and improved scientific understanding of bone and soft tissue regeneration have led to a new class of biotechnology-derived therapeutics for craniomaxillofacial (CMF) surgery. These therapies have moved beyond the original focus on BMPs to include autogenous growth factors and other recombinant human growth and morphogenetic factors. Using naturally occurring proteins and peptide sequences, rather than synthetic chemical analogs, these therapies take advantage of the body's own biological functions and processes to promote tissue regeneration in CMF surgery.

AUTOGENOUS GROWTH FACTORS

Growth factors are naturally occurring proteins that act through cell surface receptors to mediate mitogenic, chemotactic, angiogenic, and morphogenetic effects involved in the development, growth, remodeling, repair, and regeneration of tissues and organs.⁸

Platelets are a naturally occurring and easy-to-isolate source of growth factors, including platelet-derived growth factor (PDGF-AA, PDGF-AB, and PDGF-BB), transforming growth factor β (TGF- β_1 and TGF- β_2), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), epithelial growth factor, and insulin-like growth factor 1 (IGF-1),⁹ among others (Fig. 1, Table 1). Marx and colleagues¹⁰ first described the use of platelet-rich plasma (PRP)

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Possible PRP Constituents

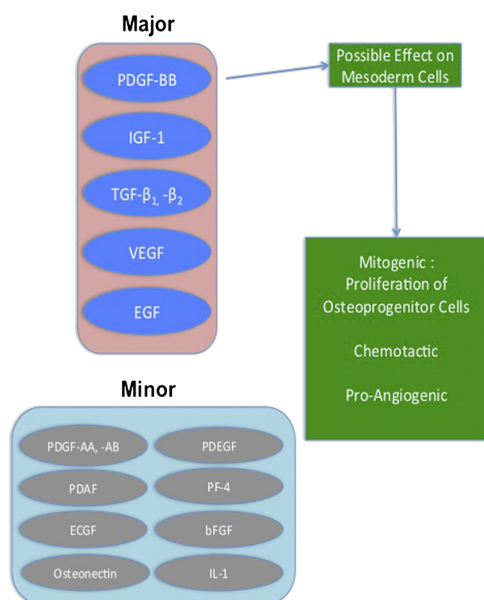


FIGURE 1. Schematic of the contribution of growth factor constituents of PRP to bone regeneration for craniofacial applications. The major growth factors (highest concentrations in PRP) and the minor growth factors (lowest concentration) are outlined. Included in the growth factors found in PRP are PDGF, IGF, TGF- β , VEGF, epidermal growth factor (EGF), interleukin 1 (IL-1), bFGF, endothelial cell growth factor (ECGF), platelet-derived angiogenesis factor (PDAF), platelet factor 4 (PF4), osteonectin, and platelet-derived epidermal growth factor (PDEGF). The individual growth factor components in PRP are known to have biological effects on mesoderm cells, and it is postulated that the combination of these activities actively contributes to bone repair.

as a source of autogenous growth factors for enhancing repair in mandibular defects in 1998. Platelet-rich plasma preparation takes advantage of the differences in buoyant density among red blood cells, platelets, and white blood cells to concentrate the platelets and white blood cells in a minimum volume of plasma by centrifugation.¹¹ This results in concentration of platelet-released growth factors to levels greater than those found in whole blood (pg/mL to ng/mL concentrations) (Table 1) in a small volume of plasma that can be delivered to the repair site. Marx¹² defined PRP as “a volume of autologous plasma that has a platelet concentration above baseline.” He further described the working definition of PRP to have a minimum of 5-fold concentration of platelets in a volume of plasma using a double-spin technique.¹² This definition is not always adhered to in the preparation of PRP, leading to an abundance of PRP preparation methods with variable growth factor compositions. The wide variability in PRP preparation methods and in the resulting growth factor composition of PRP can lead to confusion in the literature about what is actually being used in preclinical and clinical studies. Attempts to classify the different preparations of platelet concentrate have been made¹³; however, the descriptions are often used interchangeably and thus have led to confusion and controversy.

Platelet-rich plasma was reported first for the treatment of mandibular defects.¹⁰ Applications have now included craniofacial, orthopedic, and “general” wound healing. The preparation of the PRP has been made possible by a number of devices that have been approved via the 510(k) route by the US Food and Drug Administration (FDA). Characterization of the PRP produced using these devices has revealed high variability both in growth factor concentration and cellular composition (eg, leukocytes)^{14–28} (Table 1). Without proper methods to control or optimize any of the multiple variables (eg, growth factor concentrations/ratios) during PRP preparation, it re-

mains unknown which of the components are necessary, beneficial, or adverse for tissue regeneration. As a result, the outcomes of in vivo studies are applicable only to that specific preparation/delivery/indication, making it virtually impossible to compare results across studies.

Clinical Applications of PRP in CMF Indications

Platelet-rich plasma has been investigated clinically for oral and maxillofacial surgery since the early 1990s.¹⁰ Since then, many clinical studies have been performed to assess the efficacy of PRP in maxillofacial and periodontal applications. A search of the PubMed Database returns hundreds of articles for clinical studies using PRP in CMF indications. Recently, systematic reviews have been performed focusing on properly controlled, well-powered, randomized clinical trials (RCTs) investigating PRP in periodontal intraosseous defects,⁴⁰ in periodontal intrabony defects and gingival recession,⁴¹ and in dentistry⁴² (including periodontal defects, sinus augmentation, oral-maxillofacial reconstructions, and bone formation in extraction sites). These systematic reviews account for a total of 29 RCTs of high level of evidence that use a comprehensive range of PRP preparation devices, delivery systems, and controls. One important inclusion criterion of these systematic reviews was that a proper control was used for comparison, which was most often a carrier control. Of these RCTs, 17 evaluated the effect on periodontal intraosseous defects with 5 observing a positive effect and 12 finding no difference compared with control.^{40–42} For treatment of gingival recessions, 5 of 6 studies found no difference between PRP and the control treatment, whereas 1 of 6 reported a positive effect.⁴¹ In sinus augmentation procedures, 2 studies were reviewed with no overall significant difference with PRP.⁴² A total of 3 studies investigated oral-maxillofacial reconstructions, with 2 reporting positive results and 1 reporting no difference with PRP.⁴² A single study investigating bone formation in extraction sites found positive results when PRP was used.⁴² Overall, only 9 studies in oral-maxillofacial and periodontal surgery found a positive effect of PRP, whereas 20 studies found no effect when compared with the control without PRP. The authors^{40–42} all noted the considerable variability in the methods of preparation and methods of delivery of PRP and cited this as a possible factor for the heterogeneity of the outcomes. This clearly highlights the need for standardized and characterized preparations and delivery of PRP to improve the predictability of the outcomes. This also suggests that specific preparations of PRP can be effective in particular indications, but caution must be exercised in extrapolating the results to PRP treatments as a whole. An alternative to the ambiguous compositions of PRP and uncertain clinical outcomes is a therapeutic with a defined dose, well characterized, and having a known therapeutic consequence. Recombinant human growth factors fulfill these criteria.

RECOMBINANT GROWTH FACTOR TECHNOLOGIES

Recombinant DNA technology using a variety of expression systems, including yeast (*Saccharomyces cerevisiae*), bacteria (*Escherichia coli*), or mammalian (Chinese hamster ovary) cells,⁴³ allows large-scale production of highly purified analogs of human proteins for research, surgical, and pharmaceutical applications. Recombinant proteins intended for clinical use must conform to good manufacturing practices, which tightly regulate the quality and sterility of the final product, ensuring a consistent final therapeutic.

Recombinant human proteins are often referred to by their protein name, with the prefix “rh-” to signify that it is recombinantly produced using the human amino acid sequence (eg, recombinant human BMP-2 [rhBMP-2]). In addition, as with any other pharmaceutical, recombinant proteins for use in clinical applications are

TABLE 1. Respective Role of Growth Factors in Bone Regeneration and the Range of Concentrations Found in Various PRP Characterization Studies

Growth Factors	Concentration in PRP, $\mu\text{g/mL}$	Biological Effect
BMP-2 [29]	0	Osteoblast differentiation Bone maturation
BMP-7 [29]	0	Osteoblast differentiation Bone maturation
VEGF [14, 16–19, 24, 30]	$0\text{--}2.0 \times 10^{-3}$	Promotion of a vascular network Endochondral ossification
TGF- β_1 [14–20, 23–26, 28, 29, 31]	20×10^{-6} to 0.9	Osteogenesis Increases ECM production in mesenchymal cells Cell growth and differentiation of osteoprogenitor cells
PDGF-AB [15, 17–20, 23, 26–28, 31, 32]	9.7×10^{-3} to 0.3	Angiogenesis Immunosuppression Increased cell density ECM production
PDGF-BB [14, 16, 18, 24, 25, 32]	2.4 to 33.2×10^{-3}	Increased cell density ECM production Promotion of a vascular network
GDF-5 [29]	0	Increased cell density Chondrogenesis
IGF-1 [14–16, 19, 23, 25, 28, 31, 33–37]	50×10^{-6} to 0.15	Increased cell density
FGF-2 (bFGF) [16, 38]	$0\text{--}197 \times 10^{-6}$	Catabolic and anabolic effect on osteogenesis Increased cell density Angiogenesis Chondrogenesis
EGF [14, 16, 17, 24, 39]	$150\text{--}790 \times 10^{-6}$	Osseous healing Increased cell density Differentiation of osteoprogenitor cells

ECM = extracellular matrix.

assigned International Nonproprietary Names (INNs) to ensure global recognition during the regulatory process.⁴⁴

The availability of highly purified recombinant human proteins has been a valuable research tool in the discovery and understanding of mechanisms involved in craniofacial development and regeneration. These discoveries have led to the development of recombinant human growth factors as therapeutic agents to improve tissue healing and regeneration in craniofacial applications.

The regulatory process required to bring recombinant human growth factors to the clinic ensures a safe, consistent product with predictable clinical outcomes. To date, there are 2 devices composed of a recombinant growth factor with a carrier matrix approved by the US FDA for craniofacial surgery applications. GEM 21S Growth-Factor Enhanced Matrix (Luitpold Pharmaceuticals, Shirley, NY) is a combination device composed of recombinant human PDGF-BB (rhPDGF-BB) and a synthetic β -tricalcium phosphate (β -TCP) matrix. This composition is indicated to treat intrabony periodontal defects, furcation periodontal defects, and gingival recession associated with periodontal defects. The second medical device is INFUSE Bone Graft (Medtronic Sofamor Danek), a combination device composed of recombinant human BMP-2 (rhBMP-2) and an absorbable bovine type I collagen sponge (ACS). INFUSE Bone Graft (Medtronic, Minneapolis, MN) is indicated as an alternative to autogenous bone graft for sinus augmentations and for localized alveolar ridge augmentations for defects associated with extraction sockets.

In addition to these approved products, recombinant human bFGF (or rhFGF-2) has been investigated in clinical trials for the treatment of periodontal defects. Furthermore, there are a number of additional recombinant proteins that have shown promise in preclinical animal models of craniofacial surgery, suggesting that current and

future techniques in craniofacial surgery will use novel protein therapeutics to improve patient care.

Clinical Applications of Recombinant Growth Factor Technologies

Platelet-Derived Growth Factor BB

The family of PDGFs consists of 4 isoforms: PDGF-A, PDGF-B, PDGF-C, and PDGF-D. The 4 isoforms combine to form 5 biologically active homodimers or heterodimers, PDGF-AA, PDGF-AB, PDGF-BB, PDGF-CC, and PDGF-DD³² (Fig. 2). These dimers exert their biological effects through 2 cell surface tyrosine kinase receptors, PDGF receptor α and PDGF receptor β ,³² which are also dimeric and can present as homo-($\alpha\alpha$, $\beta\beta$) or heterodimers ($\alpha\beta$). The PDGF-BB homodimer is the only isoform that can activate all 3 combinations of receptors. The biological events triggered by the binding to these receptors are chemotaxis and mitogenesis of cells of mesenchymal origin, including progenitor cells, osteoblasts, and chondrocytes,^{32,45} making it the most important PDGF isoform for bone regeneration. In addition, PDGF-BB plays a role in angiogenesis by stimulating VEGF and $\alpha_v\beta_3$ integrin expression^{46–50} and is known to work synergistically with BMP-2 in bone formation⁴⁵ (Fig. 2). A recombinant human version of PDGF-BB (rhPDGF-BB; INN: becaplermin) has been produced and approved for clinical applications of periodontal regeneration.

In the periodontium, chronic inflammation is responsible for the catabolic process that leads to periodontal disease.⁴⁵ Clinical use of rhPDGF-BB has primarily focused on periodontal regeneration; however, it has also been investigated in sinus augmentations, horizontal bone augmentation, and ridge preservation applications. These studies have been recently reviewed by Hollinger et al⁴⁵ and

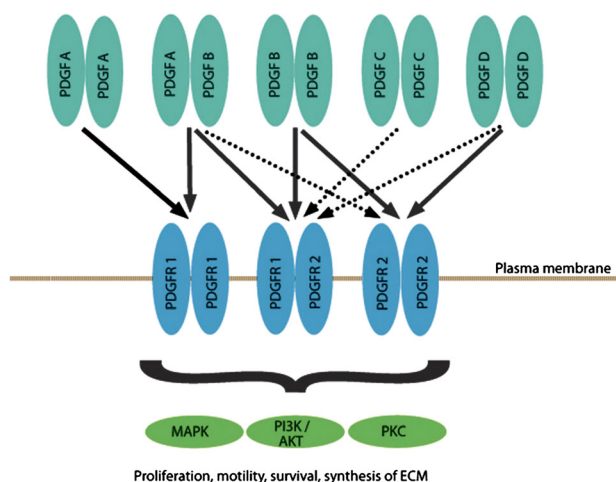


FIGURE 2. Platelet-derived growth factor isoforms signal via 2 different receptors. Platelet-derived growth factor A and PDGFB can form homodimers and heterodimers, whereas PDGFC and PDGFD form homodimers only. The receptors also form homodimers and heterodimers. Platelet-derived growth factor BB can activate all 3 combinations of receptors and is thus the most potent ligand. Intracellular tyrosine kinases phosphorylation activates signaling proteins such as phosphoinositide 3-kinase and members of the mitogen-activated protein kinase family.⁹³

Kaigler et al.⁵¹ Briefly, in separate studies, rhPDGF-BB was combined with either demineralized or mineralized freeze-dried bone allograft and applied to interproximal intrabony defects or to sites of surgical bone grafting. In both cases, rhPDGF-BB combined with allograft resulted in robust periodontal regeneration and gingival attachment. In addition to allograft, rhPDGF-BB has been combined with β -TCP to treat interproximal periodontal defects in a large randomized controlled trial of 180 patients.⁵² Treatment with rhPDGF-BB and β -TCP resulted in significantly greater clinical attachment level gain and significantly less gingival recession at 3 months and significantly increased bone fill at 6 months, results that persisted over the 24-month follow-up. Use of rhPDGF-BB has also demonstrated efficacy in soft tissue recession defects when combined with β -TCP and a collagen membrane and increased periodontal ligament attachment to cementum and alveolar bone. As such, given its role in both soft and mineralized tissue regeneration, rhPDGF-BB combined with a β -TCP matrix is a good alternative to autograft for periodontal regeneration applications.

Bone Morphogenetic Protein 2

Bone morphogenetic protein 2 is one of more than 35 TGF- β superfamily members.²⁹ Like the other members of this superfamily (discussed below), BMP-2 acts through types I and II serine/threonine kinases to induce osteogenesis of mesenchymal cells (Fig. 3). An rhBMP-2 (INN: diboterminal alfa) has been approved for clinical use for sinus augmentation and alveolar ridge augmentation.

Clinical investigations of rhBMP-2/ACS for craniofacial applications have recently been reviewed by Davies and Ochs⁵³ and Smith et al.⁵⁴ For alveolar ridge defects, treatment with rhBMP-2/ACS achieved significantly greater bone augmentation compared with controls and allowed for stable and functional placement of implants. In maxillary sinus augmentations, rhBMP-2/ACS has been used to induce sufficient bone for dental implant placement. Furthermore, a series of clinical studies have been performed to compare rhBMP-2/ACS to autogenous bone graft. In a phase 2 study,⁵⁵ the mean increase in bone height was comparable between the rhBMP-2/ACS and the autograft groups after 4 months of healing. The bone density was significantly greater in the bone graft compared with the

rhBMP-2/ACS at 4 months after surgery, but the bone density was comparable 6 months after functional loading. Following the pilot study, a pivotal clinical trial comparing rhBMP-2/ACS to autogenous bone graft in sinus floor augmentation was performed.⁵⁶ In this trial, the mean change in bone height was comparable for the 2 groups. At 6 months postoperatively, the new bone was significantly denser in the autograft group. This observation was reversed at 6 months following dental restoration, with the new bone significantly denser in the rhBMP-2/ACS group. The success rate for implants placed in the new bone was similar between groups. In addition to these approved indications, rhBMP-2/ACS has been used in alveolar cleft repairs in both children and adults. In children,⁵⁴ successful union was achieved in 49 of 50 repairs, with no negative local or systemic effects reported. In adult patients,⁵³ alveolar cleft repair with rhBMP-2/ACS resulted in more of the defect filled with new bone and a lower complication rate compared with iliac crest bone graft.

Fibroblast Growth Factor 2

Fibroblast growth factor 2 (INN: trafermin), also called bFGF, is an 18-kd protein that promotes chemotaxis and mitogenesis of many cell types, including endothelial cells, smooth muscle cells, and mesenchymal cells.³⁸ Among other functions, FGF-2 plays a role in angiogenesis, chondrogenesis, and osseous healing.³⁸ Because of these biological effects, rhFGF-2 has drawn interest as a potential therapeutic for periodontal applications. The safety and efficacy of rhFGF-2 with 3% hydroxypropylcellulose as a vehicle in periodontal regeneration have been assessed in 2 RCTs.^{57,58} In these studies, doses of rhFGF-2 ranging from 0.03% to 0.4% were applied to 2- or 3-walled vertical bone defects greater than 3 mm as measured apical to the bone crest, and patients were followed up for 36 weeks. No serious adverse events or clinical safety problems attributable to rhFGF-2 were identified. In the first study,⁵⁷ the rate of increase in alveolar bone height was significantly increased with 0.3% rhFGF-2 compared with the vehicle control; however there were no significant differences noted for gains in clinical attachment level and alveolar bone gain. In the second study,⁵⁸ rhFGF-2 showed significant superiority in the percentage of bone fill, with the percentage peaking in the 0.3% rhFGF-2 group. Once again, no significant differences in the clinical attachment regained were noted.

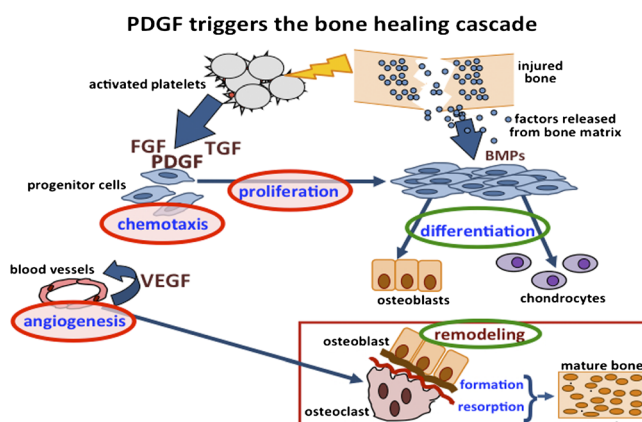


FIGURE 3. Platelet-derived growth factor and BMPs are cofactors in the bone healing cascade, working synergistically to result in formation of mature bone. Activation of platelets in the wound healing milieu results in an increase in PDGF, which induces chemotaxis and mitogenesis of osteoblasts and progenitor cells. In addition, PDGF promotes angiogenesis through the up-regulation of VEGF. Bone morphogenetic proteins released from the bone matrix induce differentiation of the progenitor cells, leading to osteoblast and chondrocyte differentiation, which facilitate the process of endochondral ossification, ultimately resulting in remodeled, mature bone.

Potential Future Recombinant Growth Factors for CMF Surgery

In addition to those recombinant growth factors that have shown clinical efficacy, other recombinant growth factors have shown promise in preclinical investigations on their effect in craniofacial applications, suggesting that they may have potential in clinical applications.

TGF- β Superfamily

The TGF- β superfamily comprises more than 35 cysteine knot proteins that include TGF- β s, BMPs, and growth differentiation factors (GDFs).²⁹ Many TGF- β superfamily members are referred to by multiple names, including osteogenic protein (OP) or cartilage-derived morphogenetic protein. These names are often used interchangeably, as evidenced by BMP-7/OP-1 and GDF-5/cartilage-derived morphogenetic protein 1. Transforming growth factor superfamily members signal through types I and II receptors, which are transmembrane serine/threonine kinases that affect gene expression through the mothers against decapentaplegic (SMAD) signal transduction pathway.²⁹ Among other functions, TGF- superfamily members are important for vasculogenesis and skeletal morphogenesis and development.^{29,59} More specifically to craniofacial applications, TGF- β s and BMPs regulate bone cell metabolism and stimulate differentiation of progenitor cells to osteochondrogenic lineage.^{29,59} In addition to BMP-2, other members of this superfamily appear to promote bone regeneration in craniofacial applications. Of note for potential craniofacial applications are TGF- β_1 , TGF- β_3 , BMP-7, and GDF-5. Of these, rhTGF- β_3 (INN: avotermin), rhBMP-7 (INN: eptotermin alfa), and rhGDF-5 (INN: radotermin) have been registered with the INN for potential clinical applications (Fig. 4).

TGF- β Isoforms (TGF- β_1 , TGF- β_2 , and TGF- β_3)

The efficacy of the TGF- β isoforms to promote bone formation in craniofacial surgery and periodontal tissue regeneration applications has recently been reviewed.^{60,61} These reviews report mixed results for bone formation following application of one of the isoforms of TGF- β . In calvarial defects, implantation of the TGF- β isoforms results in limited bone formation; however, rhTGF- β_3 has been shown to form bone in periodontal furcation defects. The best results using isoforms of TGF- β for bone formation have been observed with the addition of a cell source, such as autogenous rectus abdominus muscle tissue, or when combined with another growth factor such as OP-1. This degree of variation and therefore unpredictability in the results must be overcome before TGF- β is a viable option for clinical craniofacial surgery applications.

Recombinant Human Osteogenic Protein 1 (rhBMP-7)

Preclinical studies with rhOP-1 have included calvarial and mandibular defects in nonhuman primate and ovine models. Ripamonti et al^{62–64} performed a series of studies that suggested rhOP-1 promoted calvarial bone formation. Abu-Serriah and colleagues^{65–67} applied rhOP-1 on a type I collagen matrix to ovine mandibular osteoperiosteal continuity defects, with variable results ranging from failing to restore the original contour to filling of the defect with bone of inferior quality or mechanical properties.

Clinical use of rhOP-1, reported in case studies or small clinical trials for sinus floor elevation procedures,^{68–70} suggested outcomes similar to those observed in preclinical studies. These studies indicated that rhOP-1 has potential for initiating bone formation, although the results were inconsistent. Sufficiently powered RCTs are necessary to determine the clinical utility of rhOP-1 in craniofacial applications.

Recombinant Human Growth Differentiation Factor 5

Recombinant human GDF-5 has been used in preclinical models for periodontal regeneration. Moore et al⁷¹ analyzed the results of 22 studies for craniofacial and orthopedic applications, and the overall suggestion was enhanced local bone formation, fracture healing and repair, and cartilage, tendon, and ligament formation. The authors concluded that rhGDF-5 is a promising therapeutic agent for periodontal wound healing and regeneration. Furthermore, a number of recent animal studies have concluded that rhGDF-5, combined with carrier matrices, may increase bone and cementum formation in periodontal applications.^{72–77} Similar to rhOP-1, sufficiently powered RCTs are necessary to determine the clinical utility of rhGDF-5 in craniofacial applications.

Parathyroid Hormone

The parathyroid glands are endocrine glands that secrete parathyroid hormone (PTH). Parathyroid hormone is an 84-amino-acid polypeptide; however, the first 34 amino acids have biological activity.⁷⁸ Parathyroid hormone acts through a single G protein-coupled receptor (G protein-coupled receptor 1) to regulate mineral ion homeostasis. Parathyroid hormone affects osteoblast and stromal cell function and mediates osteoclast function through osteoblasts/osteoclast interactions.

Parathyroid hormone is commonly associated with bone resorption, although a recombinant human form of amino acids 1 to 34 of PTH (rhPTH[1–34]) (INN: teriparatide) has been approved as a treatment for osteoporosis. The paradoxical effect arises from the anabolic rather than catabolic effects that are produced by intermittent PTH administration. Based on improving bone density in osteoporotic patients, investigators have used preclinical animal models to evaluate rhPTH(1–34) to improve implant fixation, mandibular fracture healing, and bone formation in cranial defects.

Jung and colleagues^{79–81} used an arginine-glycine-aspartic acid-modified, polyethylene glycol (PEG)-based hydrogel or the PEG hydrogel combined with hydroxyapatite/TCP to deliver rhPTH(1–34) to bone defect sites surrounding titanium implants in the mandibles of dogs or in a rabbit cranial defect. The rhPTH(1–34) increased the percentage of bone formation in rhPTH(1–34)-treated defects. These studies suggest increased bone formation compared with the PEG hydrogel alone; however, there were no significant differences compared with an autograft. Investigators have also used intermittent

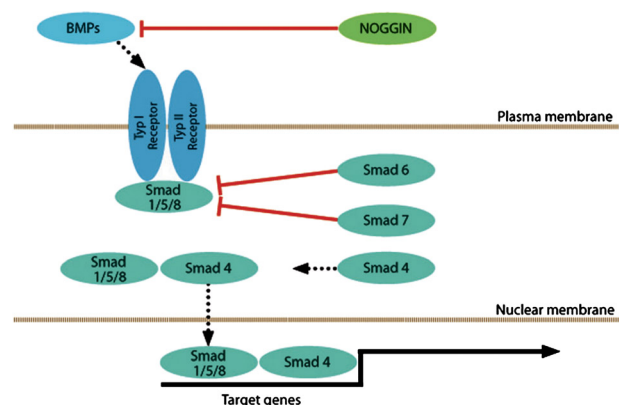


FIGURE 4. Bone morphogenetic proteins signal by binding to BMP receptor, type II (BMPRII). Bone morphogenetic protein receptor 1A or BMPRII is recruited and phosphorylates intracellular receptor-regulated mothers against decapentaplegic homologs (SMADs). SMAD4 supports signaling and activates responsive genes, whereas inhibitory SMADs (SMAD6 and SMAD7) block this process. Noggin and chordin are extracellular binding proteins that also inhibit this process at the extracellular level.⁹³

systemic delivery of rhPTH(1–34) to improve fracture or cranial bone healing. Early enhancement of healing⁸² and increased local bone formation were observed with rhPTH(1–34).^{83,84} Clinical administration of rhPTH(1–34) is currently used for osteoporosis, and a search of www.clinicaltrials.gov returns 2 clinical trials investigating the systemic administration of rhPTH(1–34) for craniofacial osseous regeneration in the oral cavity or for periodontal regeneration.

Insulinlike Growth Factor 1

Insulinlike growth factors are a family of polypeptide growth factors that are structurally related to insulin.³³ There are 2 isoforms, IGF-1 and IGF-2, and activity and half-life are regulated by IGF-binding proteins (IGFBP1–6).

Insulinlike growth factor 1 acts primarily through the IGF-1 receptor, which is expressed on muscle, cartilage, and bone cells. Insulinlike growth factor 1 upregulates genes for osteoblast differentiation. Moreover, it may have a significant impact on the expression of Osterix (Osx) and expression of Runx2.⁸⁵ These are downstream BMP transcriptional proteins associated with osteoblasts. Furthermore, IGF-1 appears to upregulate type I collagen and alkaline phosphatase.⁸⁶

A recombinant human IGF-1 (rhIGF-1; INN: mecasermin) has been produced and investigated for bone healing in preclinical animal models. Data from work by Thaller and colleagues^{87–90} suggest that IGF-1 may accelerate and improve healing in intramembranous bone defects in rats, including compromised wound healing models. Additional preclinical and clinical applications of rhIGF-1 have focused on the effect on bone regeneration in combination with rhPDGF-BB. A series of preclinical studies by Giannobile et al,⁹¹ Lynch et al,⁹² and Becker et al⁹³ indicate that the combination of rhPDGF-BB and rhIGF-1 may stimulate bone healing in canine and nonhuman primate periodontal defects. This dual relationship was confirmed in studies by Nociti et al⁹⁴ and Stefani et al⁹⁵ investigating the regeneration of bone around implants in a canine model. A phase 1/2 clinical trial also demonstrated a significant improvement in alveolar bone formation when rhPDGF-BB and rhIGF-1 were codelivered to osseous defects in a gel vehicle.⁹⁶

Vascular Endothelial Growth Factor

Vascular endothelial growth factors are a subfamily of the PDGF family that promote angiogenesis. Given the role of angiogenesis in bone healing, VEGF indirectly increases osteogenic differentiation.³⁰

Vascular endothelial growth factor A was the first member of the VEGF family, and currently 5 isoforms have been identified. Vascular endothelial growth factor A is produced by chondrocytes and osteoblasts and is regarded as a key factor in endochondral ossification.⁹⁷

Vascular endothelial growth factor ligands bind to 2 tyrosine-kinase receptors, VEGF receptor 1 (VEGFR-1) and VEGFR-2, which dimerize and become activated.⁹⁸ After the signaling proteins phosphorylate, they activate a downstream signaling cascade.⁹⁹

Vascular endothelial growth factor is chemotactic and mitogenic during the complex process of angiogenesis.^{97,100,101} Angiogenesis leads to an increase in oxygenation and other nutrients needed for bone formation.⁹⁷ A recombinant human form of VEGF (rhVEGF; INN: telbermin) has been produced and is being investigated clinically as a therapeutic for foot ulcers; however, the analysis of its effect on bone is currently limited to preclinical evaluations.

Challenges for Recombinant Growth Factors for Clinical Indications

The challenges for recombinant growth factors in craniofacial surgery are both biological and regulatory in nature. Biologically,

variables such as the delivery system and the dosing of recombinant proteins are key to optimizing the observed efficacy. A delivery system, often in the form of a carrier matrix, must be chosen to deliver a sufficient concentration of the growth factor to the local repair site with a pharmacokinetic release profile that enables a beneficial biological response. For example, certain growth factors may be more efficacious with a bolus release, whereas others require either a gradual or pulsatory release.¹⁰² Changes in the carrier matrix may alter the release profile of the growth factor.¹⁰³ Furthermore, the selection of a proper efficacious dose is important. Recombinant growth factors are delivered in doses that are orders of magnitude higher than those found in the body, with microgram to milligram quantities used. This has an effect not only on efficacy but also on the cost of the therapy. In addition, a biphasic response has been observed for many growth factors, indicating that too low or too high of a dose can affect the outcomes.^{52,58,104} As a result, preclinical and clinical dose range studies are important to establish the proper dose in humans to achieve predictable, positive outcomes. Moreover, the delivery system must provide a degree of shelter and localization of the growth factor during the destructive phases of the wound healing cascade. Failure to provide sanctuary for the growth factor during the lytic, anoxic period of the cascade threatens biological activity. Accomplishing the appropriate mixture of release and security to achieve a predictable therapeutic outcome poses a daunting challenge with biologicals.

Recombinant human growth factors must also undergo extensive safety and toxicity testing before initiation of clinical trials and before and after marketing approval by the US FDA. Because of the natural physiological role of growth factors in growth and development, the body's natural healing response, and certain disease states, recombinant proteins pose potential safety concerns that must be addressed. The International Conference on Harmonization has published guidance documents^{105,106} outlining preclinical safety testing regarding reproductive and developmental toxicity, immunogenicity, and carcinogenicity testing for biotechnology-derived pharmaceuticals to address these issues.

Of particular concern is the potential development of antibodies, particularly neutralizing antibodies, to either the recombinant or native form of the growth factor. Recombinant human proteins are produced using the human sequence, minimizing the degree of “non-self”; however, an immunologic response can occur as a result of protein aggregation or impurities in the composition of the final product.¹⁰⁷ Specific to the recombinant proteins mentioned previously, antiprotein antibodies have been reported in up to 4.5% of patients receiving rhBMP-2 and up to 41.0% (25.6% had neutralizing antibodies) of patients receiving rhOP-1.¹⁰⁸ There was also a subgroup of the autograft control group that had antibodies to rhBMP-2 (up to 0.8%) and rhOP-1 (up to 7.1% with 1.2% positive for neutralizing antibodies), suggesting that a subpopulation of naive patients can carry antibodies to these proteins.¹⁰⁸ The presence of antibodies did not correlate to patient outcomes or adverse events. It is unknown how the development of antibodies can impact recombinant growth factors meant to be delivered in a single dose, although there is potential to affect the efficacy of the therapeutic or inhibit (neutralizing) the natural occurring protein.¹⁰⁷ The latter is especially important, as it can affect future healing or potentially impact fetal development in women who become pregnant following dosing.

Because of the fact that some growth factors have been implicated in the progression or metastasis of certain types of cancer,^{101,109–113} carcinogenicity is a safety concern for therapeutic use of recombinant growth factors in the CMF complex. With the exception of PTH, the recombinant growth factors discussed in this review are intended for a single administration to a local repair site. Given the short systemic half-life of recombinant growth factors,^{114,115} it is unlikely that a single local dose would impact

oncogenesis or cancer progression; however, there is a potential risk that must be monitored through preclinical safety studies and followed in clinical trials. At this time, there have been no reports of increased cancer risk in craniofacial regeneration applications using recombinant human growth factors.

GENE THERAPY

Gene therapy is a powerful compelling tool for indications that may be considered life-threatening but less compelling for the more routine practices of tissue regeneration in the CMF complex. It is highly likely, however, that this statement could have been made 25 years ago about recombinant human growth factors. The notion that surgeons who used recombinant human growth factors were daring and reckless has been replaced with the understanding that judicious, appropriate use of recombinant human growth factors will become an indispensable component of the surgical tool kit. Gene therapy, likewise, will mature to this state.

Genes encoding for growth factors can be delivered by transferring DNA to cells at the surgical site through direct transfer of DNA or viral transduction or by modifying cells *ex vivo* and implanting them at the site of surgery.^{116,117} In addition, gene therapy may overcome the therapeutic need to deliver nonphysiological doses of recombinant protein. Specifically, local cells may be modified to deliver a therapeutic dose of biological locally, and through self-autoregulatory control, cease expression of the biological when tissue regeneration has been completed.

Preclinical and clinical studies have focused on maximizing the duration of growth factor expression, optimizing the delivery method, and minimizing patient risk.¹¹⁶ In the CMF area, gene therapy studies are still in preclinical and proof-of-concept stages. Studies by Jin et al^{118,119} suggested positive results with adenoviral vectors expressing PDGF-B. Other studies have shown enhancement of fracture repair by delivery of osteogenic genes, primarily by viral delivery.^{118–130}

The biggest challenge of any gene therapy/gene transfer approach is to ensure consistent production of the target therapeutic protein at a sufficient level for a period. As transduction and expression efficiencies improve, production of the therapeutic growth factor must also be able to be turned off to avoid potentially negative effects secondary to continued exposure. Despite all dramatic contemporary advances and often overenthusiastic expectations, the future for gene therapy in CMF surgery will require a sensible, incremental approach to build on a solid foundation of therapeutic efficacy and uncompromising safety. Consequently, special emphasis must be placed on placebo-controlled trials as well as immunologic, genotoxic, and oncogenic safety.

VISION FOR GROWTH FACTORS IN CRANIOFACIAL SURGERY

Biotechnology-derived pharmaceuticals, such as recombinant human growth factors and gene therapy, are currently the cutting edge for regenerative surgery in the CMF. Improved understanding of developmental biology and the complexity of tissue regeneration are key to the progressive development of recombinant DNA-derived human biologicals that will predictably and safely regenerate specific tissue phenotypes. Recombinant human biologicals must be administered to the tissue target in the proper therapeutic dose and at the appropriate time to match the wound regenerative cascade. Consistency and predictability of the regenerative outcome will have a profound impact on the field of craniofacial surgery. Although much of the focus of recombinant protein therapies is in the area of bone regeneration, it is reasonable to expect regeneration of craniofacial soft tissue structures will be possible. For instance, recombinant human proteins such as rhPDGF-BB, rhTGF- β_3 , and rhVEGF are either in

use or in clinical trials for their ability to improve wound healing and decrease scar formation. As the 21st century matures, recombinant human biologicals will have an overwhelming impact on the practice of craniofacial surgery.

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