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Growth Factors and Craniofacial Surgery

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ABSTRACT

The specialty of craniofacial surgery is broad and includes trauma, esthetics, 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 are 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 co-authors of this paper 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.

Keywords: Craniomaxillofacial Surgery; Growth Factors; Platelet-Rich Plasma; Platelet-Derived Growth Factor-BB; Bone Morphogenetic Protein-2
INTRODUCTION:

Craniofacial surgery addresses numerous and varied challenges in the craniomaxillary-mandibular complex. Likewise, within this complex there are a constellation of cell phenotypes and tissues. As a meaningful focus for this review, we will 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 augmentation of the bone and soft tissue in order 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 3-dimensional imaging[4]. Moreover, innovation in biomaterials that are biocompatible and biomechanically match bone have 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 pre-clinical 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’s observations that demineralized bone matrix (DBM)[7] could induce bone formation, provided the framework for biologically-derived growth and morphogenetic factors to enhance bone repair. While biological materials, such as autograft, allograft, and DBM 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 which 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-β1 and TGF-β2), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), epithelial growth factor (EGF), and insulin-like growth factor-1 (IGF-
Marx and colleagues first described the use of platelet-rich plasma (PRP) as a source of autogenous growth factors for enhancing repair in mandibular defects in 1998[10]. PRP 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 above 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. Marx defined PRP as “a volume of autologous plasma that has a platelet concentration above baseline”[12]. 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 made[13], however, the descriptions are often used interchangeably and thus have led to confusion and controversy.

PRP was reported first for the treatment of mandibular defects[10]. Applications have now included craniofacial, orthopaedic 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 (e.g. leukocytes)[14-28] (Table 1). Without proper methods to control or optimize any of the multiple variables (e.g. growth factor concentrations/ratios) during PRP preparation, it
remains 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 Craniomaxillofacial Indications:**

PRP has been investigated clinically for oral and maxillofacial surgery since the early 1990’s[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 craniomaxillofacial (CMF) indications. Recently, systematic reviews have been performed focusing on properly controlled, well-powered, randomized clinical trials (RCTs) investigating PRP in periodontal intraosseous defects[29], in periodontal intrabony defects and gingival recession[30], and in dentistry[31] (including periodontal defects, sinus augmentation, oral-maxillofacial reconstructions, and bone formation in extraction sites). These systematic reviews account for a total of 29 high level of evidence RCTs which utilize a comprehensive range of PRP preparation devices, delivery systems, and controls. One important inclusion criteria 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 to control[29-31]. For treatment of gingival recessions, 5 out of 6 studies found no difference between PRP and the control treatment, while 1 out of 6 reported a positive effect[30]. In sinus augmentation procedures, 2 studies were reviewed with no overall significant difference with PRP[31]. A total of 3 studies investigated oral-maxillofacial reconstructions, with 2 reporting positive results and 1 reporting no difference with PRP[31]. A single study investigating bone formation in extraction sites found positive results
when PRP was used[31]. Overall, only 9 studies in oral-maxillofacial and periodontal surgery found a positive effect of PRP, while 20 studies found no effect when compared to the control without PRP. The authors[29-31] 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 in order 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[32], allows large scale production of highly purified analogues of human proteins for research, surgical, and pharmaceutical applications. Recombinant proteins intended for clinical use must conform to good manufacturing practices (GMPs), 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 (e.g., rhBMP-2). Additionally, as with any other pharmaceutical, recombinant proteins for use in
clinical applications are assigned International Nonproprietary Names (INN) to ensure global recognition during the regulatory process[33].

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 two devices composed of a recombinant growth factor with a carrier matrix approved by the US Food and Drug Administration (FDA) for craniofacial surgery applications. GEM 21S® Growth-Factor Enhanced Matrix (Luitpold Pharmaceuticals) is a combination device composed of recombinant human platelet-derived growth factor-BB (rhPDGF-BB) and a synthetic β-tricalcium phosphate 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 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 basic fibroblast growth factor (rhbFGF or rhFGF-2) has been investigated in clinical trials for the treatment of periodontal defects. Further, 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 utilize novel protein therapeutics to improve patient care.

**Clinical Applications of Recombinant Growth Factor Technologies:**

**Platelet-Derived Growth Factor-BB (PDGF-BB):** The family of platelet-derived growth factors consists of four isoforms, PDGF-A, PDGF-B, PDGF-C, and PDGF-D. The four isoforms combine to form five biologically active homo- or heterodimers, PDGF-AA, PDGF-AB, PDGF-BB, PDGF-CC, and PDGF-DD[34] (Figure 2). These dimers exert their biological effects through two cell surface tyrosine kinase receptors, PDGF receptor α (PDGFRα) and PDGF receptor β (PDGFRβ)[34], which are also dimeric and can present as homo-(αα, ββ) or heterodimers (αβ). The PDGF-BB homodimer is the only isoform that can activate all three 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[34, 35], making it the most important PDGF isoform for bone regeneration. Additionally, PDGF-BB plays a role in angiogenesis by stimulating VEGF and α,β3 integrin expression[36-40] and is known to work synergistically with BMP-2 in bone formation[35] (Figure 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 [35]. 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 al.[35] and Kaigler et al.[41]. 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-tricalcium phosphate (β-TCP) to treat interproximal periodontal defects in a large randomized controlled trial of 180 patients [42]. 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 which 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 (BMP-2):** Bone morphogenetic protein-2 is one of more than 35 transforming growth factor-β superfamily members[43]. Like the other members of this superfamily (discussed below), BMP-2 acts through type I and type II serine/threonine kinases to induce osteogenesis of mesenchymal cells (Figure 3). A recombinant human BMP-2 (rhBMP-2; INN: dibotermin 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 et al.[44] and Smith et al.[45]. For alveolar ridge defects, treatment with rhBMP-2/ACS achieved significantly greater bone augmentation compared to 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. Further, a series of clinical studies have been performed to compare rhBMP-2/ACS to autogenous bone graft. In a phase II study [46], 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 to the rhBMP-2/ACS at 4 months after surgery, but the bone density was comparable 6 months post-functional loading. Following the pilot study, a pivotal clinical trial comparing rhBMP-2/ACS to autogenous bone graft in sinus floor augmentation was performed[47]. In this trial, the mean change in bone height was comparable for the two groups. At 6 months post-operatively, 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[45], successful union was achieved in 49 of 50 repairs, with no negative local or systemic effects reported. In adult patients[44], alveolar cleft repair with rhBMP-2/ACS resulted in more of the defect filled with new bone and a lower complication rate compared to iliac crest bone graft.

**Fibroblast Growth Factor-2 (FGF-2):** Fibroblast growth factor-2 (INN: trafermin), also called basic fibroblast growth factor (bFGF), is an 18-kDa protein that promotes chemotaxis and mitogenesis of many cell types, including endothelial cells, smooth muscle cells, and mesenchymal cells[48]. Among other functions, FGF-2 plays a role in angiogenesis, chondrogenesis, and osseous healing[48]. 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 (HPC) as a vehicle in periodontal regeneration has been assessed in two randomized clinical trials[49, 50]. 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 for 36 weeks. No serious adverse events or clinical safety problems attributable to rhFGF-2 were identified. In the first study[49], the rate of increase in alveolar bone height was significantly increased with 0.3% rhFGF-2 compared the vehicle control, however there were no significant differences noted for gains in clinical attachment level and alveolar bone gain. In the second study[50], 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.

**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.

**Transforming Growth Factor-β (TGF-β) Superfamily:** The transforming growth factor-β superfamily comprises more than 35 cysteine knot proteins that include TGF-βs, BMPs, and growth differentiation factors (GDFs) [43]. Many TGF-β superfamily members are referred to by multiple names, including osteogenic protein (OP) or cartilage-derived morphogenetic protein (CDMP). These names are often used interchangeably, as evidenced by BMP-7/OP-1 and GDF-5/CDMP-1. TGF-β superfamily members signal through type I and type II receptors, which are transmembrane serine/threonine kinases that affect gene expression through the mothers against
decapentaplegic (SMAD) signal transduction pathway[43]. Among other functions, TGF-β superfamily members are important for vasculogenesis and skeletal morphogenesis and development[43, 51]. More specifically to craniofacial applications, TGF-βs and BMPs regulate bone cell metabolism and stimulate differentiation of progenitor cells to osteo-chondrogenic lineage[43, 51]. 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-β1, TGF-β3, BMP-7, and GDF-5. Of these, rhTGF-β3 (INN: avotermin), rhBMP-7 (INN: eptotermin alfa), and rhGDF-5 (INN: radotermin) have been registered with the INN for potential clinical applications.

**TGF-β Isoforms (TGF-β1, TGF-β2, and TGF-β3):** The efficacy of the TGF-β isoforms to promote bone formation in craniofacial surgery and periodontal tissue regeneration applications have recently been reviewed[52, 53]. 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-β3 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.

**rhOP-1 (rhBMP-7):** Preclinical studies with rhOP-1 have included calvarial and mandibular defects in non-human primate and ovine models. Ripamonti et al.[54-56] performed a series of studies that suggested rhOP-1 promoted calvarial bone formation. Abu-Serriah and colleagues 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[57-59].

Clinical use of rhOP-1, reported in case studies or small clinical trials for sinus floor elevation procedures[60-62], 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 randomized clinical trials are necessary to determine the clinical utility of rhOP-1 in craniofacial applications.

rhGDF-5: Recombinant human GDF-5 has been used in preclinical models for periodontal regeneration. Moore and coauthors analyzed the results of 22 studies for craniofacial and orthopaedic applications[63] 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. Further, a number of recent animal studies have concluded that rhGDF-5, combined with carrier matrices, may increase bone and cementum formation in periodontal applications[64-69]. Similar to rhOP-1, sufficiently powered randomized clinical trials 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). PTH is an 84 amino acid polypeptide, however, the first 34 amino acids have biological activity[70]. PTH acts through a single G protein-coupled receptor (PTHR1) to regulate mineral ion homeostasis. PTH affects osteoblast and stromal cell function and mediates osteoclast function through osteoblasts/osteoclast interactions.
PTH is commonly associated with bone resorption, although a recombinant human form of amino acids 1-34 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 used an arginine-glycine-aspartic acid (RGD) modified polyethylene glycol (PEG)-based hydrogel or the PEG hydrogel combined with hydroxyapatite/tricalcium phosphate (HA/TCP) to deliver rhPTH(1-34) to bone defect sites surrounding titanium implants in the mandibles of dogs or in a rabbit cranial defect[71-73]. The rhPTH(1-34) increased the percentage of bone formation in rhPTH(1-34)-treated defects. These studies suggest increased bone formation compared to the PEG hydrogel alone, however, there were no significant differences compared to an autograft. Investigators have also used intermittent systemic delivery of rhPTH(1-34) to improve fracture or cranial bone healing. Early enhancement of healing[74] and increased local bone formation were observed with rhPTH(1-34)[75, 76]. Clinical administration of rhPTH(1-34) is currently used for osteoporosis and a search of www.clinicaltrials.gov returns two clinical trials investigating the systemic administration of rhPTH(1-34) for craniofacial osseous regeneration in the oral cavity or for periodontal regeneration.

**Insulin-like Growth Factor-1**: Insulin-like growth factors (IGF) are a family of polypeptide growth factors that are structurally related to insulin[77]. There are two isoforms, IGF-1 and IGF-2, and activity and half-life are regulated by IGF binding proteins (IGFBP1-6).
IGF-1 acts primarily through the IGF-1 receptor, which is expressed on muscle, cartilage, and bone cells. IGF-1 upregulates genes for osteoblast differentiation. Moreover, it may have a significant impact on the expression of Osterix (Osx) and expression of Runx2[78]. These are downstream BMP transcriptional proteins associated with osteoblasts. Furthermore, IGF-1 appears to upregulate type I collagen and alkaline phosphatase[79].

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 suggest that IGF-1 may accelerate and improve healing in intramembranous bone defects in rats, including compromised wound healing models[80-83]. 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 Lynch and colleagues[84-86] indicate that the combination of rhPDGF-BB and rhIGF-1 may stimulate bone healing in canine and non-human primate periodontal defects. This dual relationship was confirmed in studies by Nociti and colleagues[87, 88] investigating the regeneration of bone around implants in a canine model. A phase I/II clinical trial also demonstrated a significant improvement in alveolar bone formation when rhPDGF-BB and rhIGF-1 were co-delivered to osseous defects in a gel vehicle [89].

**Vascular Endothelial Growth Factor:** Vascular Endothelial Growth Factors (VEGF) are a subfamily of the PDGF family which promote angiogenesis. Given the role of angiogenesis in bone healing, VEGF indirectly increases osteogenic differentiation[90].
VEGF-A was the first member of the VEGF family and currently 5 isoforms have been identified. VEGF-A is produced by chondrocytes and osteoblasts and is regarded as a key factor in endochondral ossification [91].

VEGF ligands bind to two tyrosine-kinase receptors, VEGFR-1 and VEGFR-2, which dimerize and become activated[92]. After the signaling proteins phosphorylate, they activate a downstream signaling cascade[93].

VEGF is chemotactic and mitogenic during the complex process of angiogenesis[91, 94, 95]. Angiogenesis leads to an increase in oxygenation and other nutrients needed for bone formation[91]. 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[96]. Changes in the carrier matrix may alter the release profile of the growth factor[97]. Further, the selection of a proper efficacious dose is important. Recombinant growth factors are delivered in doses which 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. Additionally, a biphasic response has been observed for many growth factors, indicating that too low or too high of a dose can affect the outcomes [42, 50, 98]. As a result, preclinical and clinical dose range studies are important to establish the proper dose in humans in order 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 prior to initiation of clinical trials and before and after marketing approval by the FDA. Due to 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 (ICH) has published guidance documents [99, 100] outlining preclinical safety testing regarding reproductive and developmental toxicity, immunogenicity, and carcinogenicity testing for biotechnology-derived pharmaceuticals in order 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 immunological response can occur as a result of protein aggregation or impurities in the composition of the final product [101]. Specific to the recombinant proteins mentioned previously, anti-protein 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[102]. There was also a subgroup of the autograft control group which 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 naïve patients can carry antibodies to these proteins[102]. 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[101]. The latter is especially important, as it can affect future healing or potentially impact fetal development in women who become pregnant following dosing.

Due to the fact that some growth factors have been implicated in the progression or metastasis of certain types of cancer[103-107][95], 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[108, 109], 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 (rh) growth factors. The notion that surgeons who used rh- growth factors were daring and reckless has been replaced with the understanding that judicious, appropriate use of rh- growth factors will become an indispensible 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[110, 111]. In addition, gene therapy may overcome the therapeutic need to deliver non-physiological 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[110]. In the CMF area, gene therapy studies are still in preclinical and proof-of-concept stages. Studies by Jin et al.[112, 113] 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[112-124].

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 of time. 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. In spite of all dramatic contemporary advances and often over-enthusiastic
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, especial 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. While 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 like rhPDGF-BB, rhTGF-β3, 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.

REFERENCES:


FIGURE LEGENDS

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 platelet-derived growth factor (PDGF), insulin growth factor (IGF), transforming growth factor β (TGF-β), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), interleukin-1 (IL-1), basic fibroblast growth factor (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 contribute to bone repair.

Figure 2: Platelet-derived growth factor (PDGF) isoforms signal via two different receptors. PDGFA and PDGFB can form homo- and heterodimers, while PDGFC and PDGFD form homodynes only. The receptors also form homo and heterodimers. PDGF-BB can activate all three 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].

Figure 3: PDGF 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. Additionally, PDGF promotes angiogenesis through the upregulation of VEGF. BMPs
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.

Figure 4: Bone morphogenetic proteins (BMPs) signal by binding to BMP receptor, type II (BMPR2). BMPR1A or BMPR1B are recruited and phosphorylate intracellular receptor-regulated mothers against decapentaplegic homologs (SMADs). SMAD4 supports signaling and activates responsive genes, while 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].
Figure 1
Click here to download high resolution image
Figure 2

Click here to download high resolution image

[Diagram showing PDGF A, PDGF B, PDGF C, PDGF D, PDGFR 1, PDGFR 2, MAPK, PI3K/AKT, PKC, and their interactions]

Proliferation, motility, survival, synthesis of ECM
Figure 3

PDGF triggers the bone healing cascade

- Activated platelets
- FGF, PDGF, TGF

Progenitor cells

Chemotaxis

Blood vessels

Angiogenesis

VEGF

Proliferation

Differentiation

Osteoblasts

Chondrocytes

Remodeling

Osteoblast

Osteoclast

Formation

Resorption

Mature bone
Figure 4
Click here to download high resolution image
Table 1: The respective role of growth factors in bone regeneration and the range of concentrations found in various PRP characterization studies.

<table>
<thead>
<tr>
<th>Growth Factors</th>
<th>Concentration in PRP (µg/mL)</th>
<th>Biological Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMP-2</td>
<td>0</td>
<td>Osteoblast Differentiation</td>
</tr>
<tr>
<td>[43]</td>
<td></td>
<td>Bone Maturation</td>
</tr>
<tr>
<td>BMP-7</td>
<td>0</td>
<td>Osteoblast Differentiation</td>
</tr>
<tr>
<td>[43]</td>
<td></td>
<td>Bone Maturation</td>
</tr>
<tr>
<td>VEGF</td>
<td>0 to 2.0 x 10^{-3}</td>
<td>Promotion of a vascular network</td>
</tr>
<tr>
<td>[14, 16-19, 24, 90]</td>
<td></td>
<td>Endochondral ossification</td>
</tr>
<tr>
<td>TGF-β_1</td>
<td>20 x 10^{-6} to 0.9</td>
<td>Osteogenesis</td>
</tr>
<tr>
<td>[14-20, 23-26, 28, 43, 125]</td>
<td></td>
<td>Increases ECM production in mesenchymal cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cell growth and differentiation of osteoprogenitor cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Angiogenesis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Immunosuppression</td>
</tr>
<tr>
<td>PDGF-AB</td>
<td>9.7 x 10^{-3} to 0.3</td>
<td>Increased cell density</td>
</tr>
<tr>
<td>[15, 17-20, 23, 26-28, 34, 125]</td>
<td></td>
<td>ECM production</td>
</tr>
<tr>
<td>PDGF-BB</td>
<td>2.4 x 10^{-3} to 33.2 x 10^{-3}</td>
<td>Increased cell density</td>
</tr>
<tr>
<td>[14, 16, 18, 24, 25, 34]</td>
<td></td>
<td>ECM production</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Promotion of a vascular network</td>
</tr>
<tr>
<td>GDF-5</td>
<td>0</td>
<td>Increased cell density</td>
</tr>
<tr>
<td>[43]</td>
<td></td>
<td>Chondrogenesis</td>
</tr>
<tr>
<td>IGF-1</td>
<td>50 x 10^{6} to 0.15</td>
<td>Increased cell density</td>
</tr>
<tr>
<td>[14-16, 19, 23, 25, 28, 77, 125-129]</td>
<td></td>
<td>Catabolic and anabolic effect on osteogenesis</td>
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<tr>
<td>FGF-2 (bFGF)</td>
<td>0 to 197 x 10^{6}</td>
<td>Increased cell density</td>
</tr>
<tr>
<td>[16, 48]</td>
<td></td>
<td>Angiogenesis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chondrogenesis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Osseous healing</td>
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<tr>
<td>EGF</td>
<td>150 x 10^{-6} to 790 x 10^{-6}</td>
<td>Increased cell density</td>
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<tr>
<td>[14, 16, 17, 24, 130]</td>
<td></td>
<td>Differentiation of osteoprogenitor cells</td>
</tr>
</tbody>
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March 3, 2011

Professor Mutaz B. Habal, Editor-in-Chief
Plastic Surgery, 205 W. Dr. Martin L. King, Jr. Blvd Suite 103
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Director, Tampa Bay Craniofacial Center
Research Professor, University of South Florida
Tampa, FL

RE: The Journal of Craniofacial Surgery manuscript submission - “Growth Factors and Craniofacial Surgery”

Dear Professor Habal:

On behalf of my co-authors, I would like to submit our manuscript entitled “Growth Factors and Craniofacial Surgery” for consideration for publication in The Journal of Craniofacial Surgery.

This manuscript has not been published or submitted for publication elsewhere. All the authors have participated in the conceptualization and drafting of the manuscript. All authors have reviewed and agreed with the content of this manuscript. We hope that you share our enthusiasm for this article and find interest in our manuscript.

Sincerely,

Jeffrey O. Hollinger, D.D.S., Ph.D.
Professor of Biomedical Engineering and Biological Sciences