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BONE REGENERATION MATERIALS FOR THE MANDIBULAR AND CRANIOFACIAL COMPLEX

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Abstract

Due to different functional demands and vascularity, the mandibular and craniofacial complex require bone regenerating materials with physical and chemical properties that are different from long bone. However, certain common requirements to both locales must be addressed. Furthermore, reasons why the autograft and allogeneic bank bones are successful in regenerating bone need to be understood if the researcher is to develop satisfactory alternatives. The purpose of this paper is to review some of the requirements needed for bone regenerating materials for mandibular and craniofacial bone repair. In that effort, we have briefly described the autograft and allogeneic bank bones, animal wound models, quantitative assays, certain bone regenerating factors (growth factors and bone inductive proteins), and requirements of delivery systems for presenting bone regenerating factors to the osseous host bed.

Key Words: Bone, growth factors, bone morphogenetic proteins, osteogenesis, peptides, wound healing.

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Introduction

The autogenous graft is the most frequently used material for regenerating deficient bone in the mandibular and craniofacial complex; while allogeneic bank bone is the second most commonly used material. Neither the autogenous graft nor the allogeneic bank bone provide the patient with ideal results. Both modes of treatment have a sufficient number of deficiencies that warrant searching for superior alternatives. Over the millennia, there have been a diverse and sometimes curious selection of materials that surgeons have implanted into their patients. In an effort to either regenerate or replace deficient bone, metal and alloys (gold, steel, titanium) and semi-precious gems have been used; various animal products (whole or anorganic dog and cow bone; collagen) and ceramics (different stoichiometries of calcium-phosphates, calcium-carbonates, and calcium-sulfates) have been tried; and many types of partially purified proteins and recombinant proteins have been examined (demineralized bone matrix; antigen extracted, allogeneic bone; bone morphogenetic proteins). At this time, the autogenous graft and allogeneic bank bone are the most effective substances for regenerating new bone.

If researchers are to develop alternatives to the autogenous graft and allogeneic bank bone, it is important to study and to understand the biochemical and cellular mechanisms governing fracture repair and regeneration of ablative osseous wounds. Moreover, it is singularly important to understand why the autograft, despite recognized deficiencies, promotes bone regeneration to a degree that cannot be matched by any man-made material. If researchers are to be successful in developing alternatives, not only must they be mindful of complex biochemical and cellular processes promoting bone regeneration, they must evaluate potential alternatives in a hierarchy of defined animal wound models using quantitative methodology. The purpose of this paper, therefore, is to review some concepts governing bone wound repair and to highlight potential laboratory synthesized agents that may be available to surgeons as alternatives to the autograft and allogeneic bank bone. Furthermore,

this discourse will point out an animal paradigm and the quantitative methodology that we use in our laboratory for evaluating bone regeneration materials for the mandible and craniofacial complex.

Review

Fracture Repair

As a consequence of fracture, a hematoma results from bleeding within bone marrow, cortical blood supply, periosteal envelopes, and soft tissues (McKibbon, 1968). The hematoma is the first stage of fracture healing. Concurrent with the fracture hematoma is the inflammatory response. Local cell proliferation begins by day one and continues for about three days (Simmons, 1985). On the third day, mesenchymal cells condense and by day five, cartilage formation takes place. At the hematoma site, there is an abundance of class II histocompatibility cells with Ia molecules (i.e., in human: HLA-DR; Hulth, 1989). It has been postulated that certain osteoregenerative molecules have beta-microglobulin bound to the histocompatibility complex (Hulth, 1989). Therefore, the presence of these immune complexes may be a biochemical method for modulating bone induction. Cells such as polymorphonuclear leukocytes, histiocytes, lymphocytes, and mast cells migrate in time dependent waves to remove necrotic debris while endothelial cells proliferate and develop into capillaries penetrating the fracture hematoma. Within the hematoma, degranulating platelets (comprising the bulk of the hematoma) release platelet derived growth factor and transforming growth factors from their alpha granules. These polypeptides are chemoattractants and mitogens that have their initial effect on prefibroblast cell types. Fibroblast phenotypes elaborate a meshwork of loose connective tissue that contains proliferating capillary buds referred to as granulation tissue. The acidic pH and low oxygen tension within the hematoma result in the development of chondroblastic cells that elaborate hyalin cartilage which calcifies. As the vascular healing response matures and the oxygen tension increases to approximately 200 mm Hg, calcified cartilage is degraded and the healing fracture becomes replaced by pre-osteoblasts that differentiate into osteoblasts. The osteoblast cell line is thought to develop from pericytes found in the invading vascular tree (Owen, 1980). Fracture repair, therefore, proceeds in a centripetal direction through the hematoma, developing a bone-like callus that has a contour of greater mass and size than the original bone. By the process of remodeling, the original bony contour is returned. Within the first seven days of its development, fracture callus contains a high concentration of hyaluronate which is associated with the promotion of cell migration and mitogenesis of mesenchymal and endothelial cells. Mast cells contribute to neovascularization (Marks, 1986). Macrophages and monocytes release the cytokine interleukin-1 (IL-1). As yet the roles of IL-1 have not been completely determined; however, it is associated with mitogenesis of certain bone marrow cells and osteoclasts. Friedenstein described two types of osteogenic cells based on their functional properties: determined osteoprogenitor cells (DOPC) and inducible osteoprogenitor cells (IOPC; Friedenstein, 1973). Purportedly, DOPC are derived from pluripotential cells in the periosteum and marrow, whereas IOPC are derived from mesenchymal precursors that are induced to express specific phenotypes based on the inducting agent (morphogen).

The biochemical signals that herald the appearance of chondrocytes and osteoblasts remain to be elucidated. However, it is likely that proteins and polypeptides (i.e., bone morphogenetic protein, interleukins, growth factors) are released from endogenous depots, such as fracture ends and from the hematoma. While bone morphogenetic protein (BMP) has been directly implicated in bone induction, it has not been identified at the fracture callus (Brighton, 1984). Fractures bring about the release of prostaglandin E_2 (PGE_2) from the bone and muscle. While PGE_2 has osteolytic effects *in vitro*, there is putative evidence that effects *in vivo* promote bone formation (Chapman, 1987). It is likely that growth factors regulate both bone resorption and formation (remodeling). At the fracture site, transforming growth factor beta (TGF-β) is released in a latent form that is activated by proteolytic enzymes. In its activated form, TGF-β promotes conversion of mesenchymal cells into cartilage cells and it enhances production of collagen, fibronectin, and plasminogen activating factor in osteoblasts. Additional comments on TGF-β will be made later in this paper.

The embryologic origin of bone was thought to have a lasting and significant impact on the sequence of fracture healing. Endochondrally derived long bone repairs fractures through a cartilaginous process, whereas intramembranous bone fractures lack a cartilaginous phase. The literature taught that maxillary fractures did not heal by osseous union because the maxilla lacks a periosteum (Brayshaw, 1947). However, there have been animal studies refuting this clinically derived assumption and a recent human study has dispelled the notion of fibrous healing of the mid-face fracture (Thaller and Kawamoto, 1990).

Regulatory chemical messengers of fracture repair can be classified as autocrine (the synthesis and target cells are one in the same), paracrine (the synthesis cell product diffuses to a different responding cell), and endocrine (the synthesis product transits via the blood from source cell to target cell; Sporn and Todaro,

1980). Transforming growth factor beta (TGF-β) is both an autocrine and paracrine cell modulator. Macrophages release TGF-β which affects fibroblasts and endothelial cells. Insulin-like growth factors 1 and 2 (IGF-1 and 2) are examples of endocrine cell modulators. The cell modulators (chemical messengers) are peptide to polypeptide sized moieties whose primary attributes are to promote chemoattraction and mitogenesis. The regulatory chemical messengers associated with callus formation at the fracture are platelet derived growth factor (PDGF) (mitogenic for fibroblasts and bone cells; activates monocytes and promotes bone resorption); epidermal growth factor (EGF) (mitogenic for chondroblasts and osteoblasts; inhibitory for type I bone collagen, and promotes bone resorption); fibroblast growth factor (FGF) (mitogenic for fibroblasts, chondroblasts and osteoblasts); TGF-β (a mitogen to osteoblasts and augments collagen synthesis); IGF (enhances chondrocyte proliferation, proteoglycan synthesis and collagen synthesis); nerve growth factor (NGF) (a differentiation maintenance factor); and the monocytic interleukins (IL-1, a potent bone resorption factor, which enhances fibroblast proliferation, collagenase and prostaglandin production; and IL-2, which may enhance T-cell growth factor and aid in bone resorption; Hauschka 1990). Despite the compelling evidence for the action of the autocrine, paracrine, and endocrine factors, one needs to be mindful that most data are based on *in vitro* actions in isolated cell preparations. That growth factors are operant in fracture healing and repair needs to be determined by immunohistochemical and molecular biologic methods. Moreover, strict attention needs to be paid to the biochemical-chronopharmacologic effects of the growth factors. Because of the importance of these chemical messengers, additional comments later in this paper will be directed at their relevancy to bone regeneration.

Medullary callus repair studies indicate that capillary and venous endothelial cells in the fracture region enlarge, polymorphic mesenchymal cells stream to the fracture site and become abundant throughout the medullary callus, and osteoblasts and bone formation occurs 24 hours post-fracture (Heiple *et al.*, 1987). There is a suggestion that endothelial cells, reticular cells, blood vessel pericytes, and polymorphic mesenchymal cells may be interrelated and either may be osteoblast progenitor cells or may in some manner lead to phenotypic expression of osteoblasts.

Bone Graft Repair: Autograft

The cellular and biochemical events at the graft-host interface are a reiteration of fracture repair. During the first seven days, the hematoma and inflammatory response is characterized by vascular invasion, the appearance of inflammatory exudate is typified by such cells as lymphocytes, polymorphonuclear leukocytes (neutrophils), and plasma cells. Between 7-14 days, granulation tissue develops, giant cells and osteoclasts populate the graft host bed, and angiogenesis commences. During the first 14 days, there is little difference between the cancellous versus cortical autograft. Revascularization of the cancellous graft may occur within hours after transplantation due to anastomoses with host and graft vessels. In contrast, cortical grafts revascularize at a slower pace. The cortical graft is eroded in a centripetal fashion by the cutting cone of osteoclasts; therefore, by 14 days post-transplantation, the cortical graft is pockmarked with erosion channels. The ingress of cutting cones begins to subside by six weeks; however, cortical block grafts are weaker in physical properties than native bone. From the sixth week until six months, block grafts may be 40% to 50% weaker than host bone (Burchardt, 1987). By the first to second year after transplantation, porosity diminishes and the graft may be as strong as natural bone. The cortical graft may be admixed with host bone for the lifetime of the recipient, whereas cancellous grafts are completely replaced by regenerated host bone.

Autograft repair is in small part dependent upon the contribution of transplanted osteoprogenitor cells. A variable number of osteocytes survive the grafting procedure. Cells of the cambial layer of the periosteum can convert to preosteoblasts under the influence of inductive factors present in the graft and the host bone stump ends. Moreover, endosteal and marrow cell elements contribute inducible and determined cell populations that develop into preosteoblasts phenotypes. As the graft cells produce their protein products, bone regeneration progresses in a centripetal direction through the graft. Bone ingrowth into the graft is termed osteoconduction; while the conversion of inducible cells into osteoblast phenotypes is referred to as osteoinduction. The internal architecture of the autograft affords appropriate spatial dimensions to allow for neovascularization, cell anchorage, and proliferation to occur, thereby insuring for optimal osteoconduction and osteoinduction.

Bone Graft Repair: Allograft

In general, allografts are not incorporated as well in the host bed as autografts. The differences in large part are due to histocompatibility antigens derived from the cell surface contaminants of the allograft. As a consequence of histoincompatibility, allografts may be completely rejected and resorbed at the host bed. The consequence is fibrous union and soft tissue prolapse rather than bone regeneration. The modes of action of a successful allograft reside in the osteoconductive and osteoinductive principles. Allografts do not contribute a via-

ble cell population for the development of osteoblasts. Depending upon the degree of host acceptance of the allograft, during the first two weeks there is a typical inflammatory phase. This phase may be chronic, lasting upwards of a year. While new bone formation may be seen at the host margins of an autograft within a week post-transplantation, there may be a delay with the allograft. If the allograft is accepted by the host, most all phases of bone repair are substantially retarded as compared to the autograft (Burchardt, 1987).

Animal Paradigms Used to Study Bone Regeneration Materials

Alternatives to the graft and allogeneic bank bone must be assessed in bony wounds that do not heal by spontaneous bone formation. An intraosseous defect that fulfills this criterion is known as a critical size defect (CSD). If a bone regeneration material (BRM) is placed into a CSD and the defect goes to bony union, the bony union may be attributed to the capacity of the BRM to initiate the cascade of chronobiological events at the appropriate tempo to promote osteanaphysis. Our laboratory advocates a series of animal models for assessing BRMs that include rats, rabbits, dogs, and non-human primates (Hollinger and Kleinschmidt, 1990; Schmitz and Hollinger, 1986). We emphasize the importance of CSDs as a means to standardize comparisons of BRMs between laboratories. Animal paradigms using intraosseous CSDs have been reviewed (Hollinger and Kleinschmidt, 1990; Schmitz and Hollinger, 1986). It is strongly suggested, therefore, these reviews be consulted prior to embarking upon extensive BRM studies.

Quantitative Methods to Evaluate Bone Regenerating Materials

Quantitative evaluation of bone formation can be accomplished using computer imaging of histologic slides and X-ray films. The beauty of quantitative histology (histomorphometry) and quantitative radiology (radiomorphometry) is that the elements of bone formation can be objectively tallied and reviewed in a manner that can be reproduced by various laboratories. The scanning electron microscope (SEM) with backscatter detection has become an invaluable tool to bone researchers who evaluate bone growth in ceramic materials. Likewise, X-ray microanalysis and SEM can be applied to detection of bone formation across a CSD treated with bone regeneration compounds. Both SEM with either X-ray microanalysis or backscatter detection have been discussed in reviews and have been applied as research tools to investigate bone regenerating agents (Hollinger and Kleinschmidt, 1990). Biochemically, histochemically, immunohistochemically, and using *in situ* hybridization technology the modern bone researcher can evaluate and measure the biochemical, cellular, and stromal events of the bone regeneration cascade. Our laboratory strongly advocates applying modern, quantitative methods to assessing bone regenerating materials.

Bone Regenerating Factors: Demineralized Bone Matrix, The Bone Morphogenetic Proteins, Growth Factors

Preface

The major phases of new bone formation include migration of progenitor cells, mitosis of mesenchymal stem cells, differentiation to cartilage and bone, mineralization and remodeling, and hematopoietic marrow differentiation. The initiation of the bone formation cascade is triggered by a bone inductive protein(s) (Reddi *et al.*, 1988) and proliferation of progenitor cells is modulated by a number of growth factors (Canalis *et al.*, 1988). Bioactive proteins such as bone morphogenetic proteins (BMP) induce the differentiation of pluripotential cells into cartilage-forming cells and bone-forming cells (Urist, 1965). Several osteoinductive proteins (Sampath *et al.*, 1987; Urist *et al.*, 1984) and growth factors (Hauschka *et al.*, 1986) have been isolated from bone using different purification procedures. Some have been found to have considerable amino acid sequence homology. A number of growth factors involved in wound healing are believed to be relevant in bone remodeling. These growth factors are surmised to act in concert with osteoinductive protein, thereby augmenting osteanaphysis. Recently, a family of seven BMPs has been cloned by recombinant DNA technology and expressed. The members include BMP-1, BMP-2, BMP-3 (osteogenin: OG), BMP-4, BMP-5, BMP-6, and BMP-7 (osteogenic protein-1). The availability of recombinant BMPs will allow investigations of the mechanism of their actions and plausible clinical applications. The following brief review examines bone derivatives, inductive protein, and growth factors that may be relevant for craniofacial bone regeneration.

Demineralized Bone Matrix

Repair of craniomaxillofacial osseous defects with demineralized bone matrix (DBM) has been demonstrated (Glowacki *et al.*, 1982; Mulliken, 1982). The sequence of events of induced bone development was described by Urist using demineralized cortical block matrix (Urist, 1965; Urist *et al.*, 1973). When DBM is implanted subcutaneously in allogeneic recipients, it releases factors which act as local mitogens to stimulate proliferation of mesenchymal cells (Rath and Reddi, 1979). Mesenchymal cells differentiate by day five to chondroblasts, to chondrocytes by day seven, and to osteoblasts by day eleven. The angiogenesis that ensues by day nine has been correlated with chondrolysis.

Considerable new bone formation occurs between days 12 and 18. By day 21 an ossicle develops, complete with hematopoietic marrow lineages (Reddi, 1984).

Bone Morphogenetic Protein

Although DBM appeared to be promising, the osteoinductive potential of DBM may be limited by the quantity of DBM that can be surgically placed into the recipient bed to produce a therapeutic effect. Optimization and augmentation of osseous induction has been attained by extracting bone inductive proteins, such as, OG from DBM (Sampath *et al.*, 1987). OG was isolated by heparin affinity chromatography and preparative gel electrophoresis and the bone inductive activity was localized to the zone between 30-40 kiloDaltons apparent molecular mass. The amino acid sequence of tryptic peptides of OG was similar to BMP-3. Recently, BMP-2A, BMP-2B, and BMP-3 have been cloned and expressed (Wozney *et al.*, 1988). BMP-2A is referred to as BMP-2; whereas BMP-2B is known as BMP-4. BMP-3 has been designated osteogenin (Luyten *et al.*, 1989). In addition, a novel gene for osteogenic protein-1 (BMP-7) has been cloned (Özkaynek *et al.*, 1990). The expanding list of novel BMPs now includes BMP-5, BMP-6 and BMP-7 (Celeste *et al.*, 1990). The amino acid sequence of BMPs shows homology of the carboxy terminal quarter domain and is shared by transforming growth factor-b (TGF-β), thereby categorizing the BMPs as part of the TGF-β supergene family.

Dose-response and time-course studies have been done using highly purified and characterized human recombinant BMP-2 (derived from Chinese hamster ovary cell line; Wong *et al.*, 1990). *In vivo* bone induction was observed following implantation of the recombinant protein. Using the rat ectopic bone formation assay, implantation of partially purified recombinant human BMP-2 up to 115 μgs resulted in cartilage and bone formation by days 7 and 14, respectively. High doses of the protein induced bone formation as early as five days following implantation and the histological examination of the newly formed cartilage and bone did not reveal any significant differences when compared to DBM. cDNA clones encoding human BMP-5, BMP-6, and BMP-7 have been isolated. In addition, the effects of human recombinant BMP-1, BMP-2 and BMP-3 were examined on alkaline phosphatase activity, collagen synthesis and DNA synthesis in cultured osteoblastic cells (3MCT3-E1). These BMPs were found to stimulate the expression of osteoblastic phenotype markers but had no effects on DNA synthesis in the cultured cells. In a recent study, human recombinant BMP-2 was used with a significant degree of success in mandibular reconstruction (Toriumi *et al.*, 1991).

Growth Factors

Soft tissue wound healing and osseous regeneration are believed to involve an analogous sequence of cellular events and the cascade of events is largely governed by locally generated factors that regulate processes leading to regeneration of damaged tissue. Growth factors stimulate cell replication. Growth factor receptors are intrinsic to all cells capable of replicating. Therefore, growth factors and their receptors are prevalent in thriving tissue. Several growth factors are chemotactic for cells required for tissue regeneration (Canalis *et al.*, 1988; Leibovich and Wiseman, 1988). Following injury, platelet-derived growth factor (PDGF; Canalis *et al.*, 1988), TGF-β (Reddi, 1984) and epidermal growth factor (EGF; Canalis *et al.*, 1988; Davidson *et al.*, 1988) are released from the alpha granules of degranulating platelets. The literature suggests that the five growth factors which have the greatest potential to augment bone regeneration *in vivo* include PDGF, TGF-β, EGF, insulin-like growth factor-I (IGF-1), and basic fibroblast growth factor (bFGF; Hauschka 1990). These growth factors either have been found in bone or are capable of stimulating bone cells and tissues in a variety of *in vitro* and *in vivo* studies. It is possible that the five growth factors outlined below may be candidates for use in bone regeneration materials. Sufficient information is not available at this time to determine whether they can be used alone or if they will have to be used in combination with bone inducing protein to produce optimal bone regeneration.

PDGF

PDGF is perhaps the most abundant growth factor in serum, originating from platelet alpha granules. It exhibits chemotactic activity for monocytes, smooth muscle cells, and fibroblasts, where the AB and BB forms are more active than AA homodimer. In mitogenic assays, PDGF AB and BB forms are equipotent, while the homodimer is almost ineffective. Using bone organ culture, PDGF was shown to stimulate cell replication, collagen synthesis and non-collagen protein synthesis (Leibovich *et al.*, 1988). PDGF has been shown to augment fibroblast proliferation needed for the formation of connective tissue, smooth muscle cells and endothelial cells required for neovascularization (Leibovich and Wiseman, 1988). The mechanisms of action for these processes remain obscure. In general, there is strong evidence that PDGF is a competence factor. PDGF is synthesized by osteoblasts and is stored in bone matrix. PDGF is active in wound healing and there is evidence that it stimulates bone repair. PDGF attracts smooth muscle cells and fibroblasts to the wound sites. In older rats, this growth factor has been shown to augment demineralized bone matrix-induced heterotopic

cartilage formation which was assessed by production of mRNA for type II collagen and bone formation, measured by alkaline phosphatase and calcium levels of the implants (Hauschka, 1990). Interestingly, the effect was greater in older rats than younger ones, suggesting an inadequacy of, or decreased response to PDGF in older animals.

TGF-β

TGF-β (apparent molecular mass of 25 kiloDaltons) was originally characterized from human platelets, human placenta, and bovine kidney. It is comprised of two identical subunits cross-linked by disulfide bonds. Two cartilage induction factors, CIF-A and CIF-B, now known as TGF-β_1 and TGF-β_2 were found to have related amino-terminal sequences. Other factors whose amino acid sequences place them in the TGF-β family include inhibin, the transcript of the decapentaplegic gene complex in Drosophila, and the BMPs. It is interesting to note that the amino acid sequence of TGF-β is almost identical in a variety of species, including man, mice, chickens, cows, monkeys and pigs (Sporn and Roberts, 1989). TGF-β is known for its antiproliferative effects on cells, particularly epithelial cells, but inhibition is also common for mesenchymal cell such as fibroblasts and endothelial cells. In several cases, the antiproliferative effects correlate with augmented cellular differentiation and it has been suggested that the antiproliferative effect appears to operate distal to the receptors for other growth factors. TGF-β has been shown to have both stimulatory and inhibitory effects on proliferation of cultured osteoblasts in different studies (Hauschka, 1990).

TGF-β is known to be crucial for wound healing. The growth factor has been shown to augment two processes required for normal healing: collagen formation by fibroblasts and angiogenesis (Hauschka, 1990). Bone cells synthesize this growth factor and store it in an inert form in the extracellular matrix, thus making bone the most abundant source of TGF-β in the body. The growth factor is transformed to its active form under acidic conditions, such as those produced either during bone resorption or by macrophages. Active TGF-β converts mesenchymal cells into cartilage cells. It augments production of collagen, fibronectin, and plasminogen activating factor in bone cells that are needed for optimum regeneration of bony tissue. Both TGF-β_1 and TGF-β_2 appear to act interchangeably in most systems, but a specific role has been demonstrated for TGF-β_2 in muscle induction in the embryo. TGF-β_1 is most evident in focal areas of epithelial-mesenchymal interactions during periods of morphogenesis and remodeling. TGF-β appears to be involved in bone remodeling where it augments resorption and formation of PGE_2, a powerful stimulator of resorption, and augments local bone turnover in tissue culture. Work by Beck *et al.* have shown that TGF-β_1 promotes calcification within cartilage defects in rabbit ears following the removal of the perichondrium (Beck *et al.*, 1991a). In addition, Beck *et al.* have shown that in 12-mm diameter craniotomy defects in rabbits, 2 mg of human recombinant TGF-β_1 in 3% methylcellulose gel causes osteanaphysis by 28 days post-operation (Beck *et al.*, 1991b).

EGF

EGF, a 6.0 kiloDaltons polypeptide, contains 53 amino acid residues and 3 intramolecular cross-links. Osteoblast-like cells appear to express the EGF receptor, thereby being candidates for modulation by EGF. Receptors for EGF have been located on other bone cell types, including a macrophage-like cell and a cell type similar to undifferentiated stem cells. EGF is mitogenic for cells of both ectodermal and mesodermal origin (including osteoblasts; Davidson *et al.*, 1988; Hauschka 1990). Using reverse transcriptase polymerase chain reaction technology, it was shown that cultured embryonic ectomesenchyme of the developing mandible produced mRNA for EGF in a time-dependent fashion (Snead *et al.*, 1989). Various effects of EGF in bone organ cultures include mitogenic stimulation of periosteal fibroblasts and osteoblasts, decreased synthesis of type I collagen and alkaline phosphatase. Effects on osteoblastic cells include augmented DNA synthesis, decreased collagen synthesis, increased PGE_2 synthesis, altered intracellular calcium and increased collagenase and collagenase inhibitor synthesis (Cohen, 1962). EGF lowers the responsiveness of osteoblastic adenylate cyclase to PTH, similar to the findings for other growth factors. The ability of EGF to stimulate soft tissue wound healing is well known. In a recent study it was used with donor skin graft sites in man where it considerably decreased the healing time (Davidson *et al.*, 1988).

IGF-1

IGF-1, a 7.6 kiloDaltons polypeptide consists of 70 residues in a single chain with 3 internal disulfide bonds. The name for the growth factor was based on its ability to augment some biochemical reactions controlled by insulin, such as liver glycogen synthesis and bone collagen synthesis. It is also known as somatomedin C. Skeletal growth factor was found to be identical to IGF-2, which is homologous to IGF-1. Biological activities of IGF-1 can be regulated by several known binding proteins. The growth factor was originally thought to be a systemic growth factor regulated by growth hormone but it is clear that IGF-1 is produced by a number of tissues. It has been shown to be produced by isolated osteoblasts, cultured calvariae and osteoblast-like cells, and cartilage,

where IGF-1 regulates metabolism of proteoaminoglycans in a steady-state (Hauschka, 1990). IGF-1 was shown to stimulate synthesis of DNA, collagen and non-collagen protein in cultured rat calvariae (Canalis, 1980). The growth factor augments cell proliferation and collagen synthesis in cultured osteoblast-like cells and in the layer of immature cells adjoining the periosteum and periosteum-free calvariae. Studies using infusion of growth hormone and IGF-1 in rats implicated somatomedin production to play an important role in endochondral bone growth. There is evidence that indicates that longitudinal bone growth is regulated by growth hormone via stimulation of IGF-1 production (Hauschka, 1990).

FGF

FGF is found as both an acidic and basic form, representing a family of related growth factors apparently resulting from gene duplication and evolutionary divergence from a common ancestral protein. The growth factor is a single chain polypeptide containing 146 amino acids with apparent molecular weight ranging from 16-18 kiloDaltons. Because of its affinity for heparin, it is also called heparin binding growth factor, type 2 (Davidson *et al.*, 1988). Basic FGF (bFGF) is mitogenic for a variety of cells, including fibroblasts, chondrocytes, osteoblasts, myoblasts, smooth muscle cells, glial cells and endothelial cells. In addition to its mitogenic effect, bFGF increases osteocalcin content of conditioned media, suggesting that bFGF modulates function of osteoblast-like cells. The growth factor is synthesized by macrophages, endothelial cells, bone cells and cultured calvariae from which it is secreted into the extracellular matrix (Hauschka, 1990). It binds loosely to heparin-sulfate proteoglycans. It has been isolated from DBM by guanidine extraction followed by heparin-sepharose affinity chromatography. The growth factor has been proposed to be involved in bone remodeling. When a single injection of 150 ng of bFGF was given to a subcutaneous wound model, it was as effective as continuous infusion of 100 ng EGF/day. These data suggested that bFGF acts as a "competence" factor (Davidson *et al.*, 1988). An interesting activity of the factor is its ability to substitute with high degree of specificity for the morphogenetic action of the ventrovegetal factor in Xenopus development. bFGF augmented angiogenesis when infused into graft sites of mandibular ramus and body in rabbits (Canalis, 1980). Increase in concentrations of calcium and PGE_2 in media have been noted when the growth factor was added to the culture media of neonatal mouse calvariae. In chondrocytes, interleukin-1-mediated proteinase release can be greatly augmented by bFGF (Hauschka, 1990).

Delivery Systems

The regeneration of bone of the mandibular and craniofacial complex (or of any skeletal tissue) may be possible if a delivery system is constructed having appropriate chronobiologic properties and architectural specifications. Specifically, the architectural design should support bone ingrowth (osteoconduction) from host bone margins. Specifications for osteoconduction must support the progression of angiogenesis and subsequent neovascularization. Therefore, an average pore size should be approximately 200 - 400 mm (Ohgushi *et al.*, 1990; mimicking the average haversian system where osteocytes are no greater than 300 mm from the central haversian canal). In our laboratory, we advocate attaining the maximal pore density possible to optimize cell ingrowth, neovascularization, and osteoconduction. It must be remembered that the requirement for strength from the delivery system does need to be equivalent to bone. The capacity to maintain spatial orientation of the bone fracture or bone fragments will be the distinction of the fixation device, not of the delivery vehicle. In addition to the architectural requirements, the vehicle must afford optimum opportunity for cell attachment. Normal pluripotential cells are anchorage dependent; therefore, an attachment platform is needed by these cells before they may be modulated into preosteoblast phenotypes. Once cells attach to a substratum (the biodegradable carrier), cells must interact with that carrier to allow for appropriate spatial adaptation leading to signal transduction and gene expression of type I bone collagen, alkaline phosphatase, and the polypeptide soup needed for bone regeneration. Furthermore, the chronobiological dependency of the healing continuum requires that the appropriate quantity of bone inductive protein and growth factors are released at a therapeutic dose at the proper point(s) in time to push the bone formation cascade to completion: the regeneration of lost form and function. The delivery vehicle must be programmed with the exacting release kinetics calibrated to local requirements of the osseous tissue to be regenerated. Mandibular and craniofacial locales do not have the same vascular supply and functional demands that a long bone site will have. Consequently, the design for a bone regenerative material (BRM) must be site-specific. Furthermore, soft tissue prolapse into an ablative wound must be prevented. In addition to deploying the appropriate payload by dose and time, the delivery vehicle must occupy the ablative segments long enough to allow bone regeneration but not so long as to retard that regeneration. Also, the BRM must maintain mass to prevent soft tissue prolapse.

Applied bone research focused on the development of alternatives to autografts and allogeneic bank bone

can be successful if basic fundamentals of bone regeneration physiology and bone homeostasis are followed. This paper reviewed several important principles that could be useful in that quest.

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Discussion with Reviewers

Reviewer II: In the discussion of animal paradigms, please provide a critical evaluation of various models.
Authors: For more details, please see the previous reviews: Hollinger and Kleinschmidt (1990) and Schmitz and Hollinger (1986).

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