Current Concepts of Bone Tissue Engineering for Craniofacial Bone Defect Repair

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Abstract

Craniofacial fractures and bony defects are common causes of morbidity and contribute to increasing health care costs. Successful regeneration of bone requires the concomitant processes of osteogenesis and neovascularization. Current methods of repair and reconstruction include rigid fixation, grafting, and free tissue transfer. However, these methods carry innate complications, including plate extrusion, nonunion, graft/flap failure, and donor site morbidity. Recent research efforts have focused on using stem cells and synthetic scaffolds to heal critical-sized bone defects similar to those sustained from traumatic injury or ablative oncologic surgery. Growth factors can be used to augment both osteogenesis and neovascularization across these defects. Many different growth factor delivery techniques and scaffold compositions have been explored yet none have emerged as the universally accepted standard. In this review, we will discuss the recent literature regarding the use of stem cells, growth factors, and synthetic scaffolds as alternative methods of craniofacial fracture repair.

Keywords
- fracture
- mandible defect
- synthetic implant
- bone regeneration

Approximately 400,000 individuals present to the emergency department in the United States annually with facial fractures with the most common sites of injury being the mandible and nasal bone.[1] Recent reports suggest that the incidence of maxillofacial bony trauma continues to rise.[2] Total annual cost of treating these fractures in the United States is estimated to be over 1 billion dollars.[1] Severe traumatic injuries can be associated with significant soft tissue and bone loss, which require a more complex reconstructive approach. Large bony defects of the facial skeleton are also seen after resection of head and neck malignancies. Significant attention has been devoted to refining current methods and developing novel methods of repairing injury or bone loss within the facial skeleton.

Traditional means of repair of bony defects of the craniofacial skeleton include bone grafting, rigid fixation, and microvascular free tissue transfer for larger defects. While these current methods work well for smaller fractures and defects, the methods for larger reconstructive problems carry significant morbidities and are not always successful. Biologically compatible implants have been studied as a means of augmenting the body's natural ability to regenerate healthy bone in the craniofacial skeleton and have the potential to decrease the morbidity associated with larger reconstructive procedures.

Osteoconduction, osteogenesis, and osteoinduction are the three mechanisms needed to act together to regenerate osseous defects. Efficacious bone tissue engineering requires

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some combination of a sound osteoconductive scaffold, vascularization, appropriate intercellular signaling, and the presence of osteoblastic cells. Stem cells are the primary source for osteoblastic cells, and require activation by osteoinductive factors for new bone formation.[3–5] Stem cells may be harvested and induced to differentiate into osteoblasts either in vitro or in vivo.[6] Proangiogenic and osteogenic growth factors can be loaded in biosynthetic scaffolds before implantation into bony defects inducing native stem cells to differentiate into osteoblasts. We aim to review the use of these techniques to stimulate craniofacial repair, their potential drawbacks, and future investigations needed to optimize these strategies for use in the clinical setting.

Neovascularization

Bone healing requires the process of neovascularization, which involves both vasculogenesis and angiogenesis. Bone tissue engineering has focused on enhancing these processes by engineering provasculogenic stem cells and by creating functional substitutes for native periosteum, which stimulates angiogenesis. Further research is needed to refine these techniques for clinical use.

Vasculogenesis and angiogenesis are essential components of bone healing. They fall under the broader category of neovascularization, a process by which an organ creates new blood supply when met with a greater blood demand (i.e., in ischemic tissue). Angiogenesis involves proliferation of local endothelial cells to produce new blood vessels from preexisting vessels in a remodeling process. Vasculogenesis, on the other hand, involves the differentiation of in situ endothelial cells into de novo blood vessels and can include the migration of bone marrow-derived adult stem cells to the site of interest via the systemic circulation.[5]

Musculoskeletal trauma is known to cause a systemic vasculogenic response.[5] This response involves the release of angiogenic factors, such as vascular endothelial growth factor (VEGF). VEGF, in turn, causes further release of other cytokines and growth factors, which ultimately cause proliferation and mobilization of adult stem cells (ASCs). Circulating endothelial progenitor cells can be detected within 6 hours following trauma. Their concentration in blood is directly proportional to VEGF levels.[6] These cells are recruited to ischemic sites where they contribute to angiogenesis.[5]

As vasculogenesis is tightly linked to osteogenesis, Wang et al hypothesized that bone healing could be augmented by increased levels of circulating progenitor cells. In their study, calvarial defects were created in the mouse model. One group was exposed to a hematopoietic stem cell mobilizer, AMD3100 (Genzyme, Cambridge, MA), which brought circulating stem cells to supraphysiologic levels. They showed increased bone stock and angiogenesis both radiographically and histologically in the group with higher levels of circulating progenitor cells.[5]

Vasculogenesis is also critical for ensuring the viability of biosynthetic implants. Techniques including tissue engineering of blood vessels, introduction of cells containing proangiogenic factors, and local delivery of angiogenic factors augment vasculogenesis.[7] Given the role of the periosteum in stimulating osteogenesis and angiogenesis, Elbackly et al hypothesized that functional periosteal substitute would stimulate blood vessel growth. Bone marrow stem cells were placed within a platelet-rich plasma (PRP) gel membrane.[7] This resulted in migration of endothelial cells, induction of osteogenic mediators, including bone morphogenetic protein-2 (BMP-2), Runx2, and osteocalcin, and significantly increased levels of proangiogenic mediators including interleukin (IL)-8, platelet-derived growth factor-BB, and VEGF. Given the dependence of osteogenesis on an adequate vascular network, the authors theorized that the induction of osteogenic mediators and endothelial cell migration was facilitated by the creation of a proangiogenic environment within the PRP gel membrane.

Evidence suggests that vasculogenesis may, in fact, be the first step in promoting osteogenesis.[8] Herring et al studied the periosteal vasculature in a pig model and analyzed the vascular and osteogenic architecture of the temporal and zygomatic bones. They labeled the extracellular matrix with calcein dye and injected a vascular fill into piglets at 2 to 6 weeks of life. Calcein binds to calcium phosphate in osteoblasts and is useful for quantifying in vitro mineralization.[9] The calcein-labeled matrix was mineralized in the last 3 hours of the pig's life. They then compared the labeled matrix to the previously existing periosteal vasculature.[8] Bone developed around the blood vessels, indicating that the pattern of neovascularization dictates subsequent bone growth. If this is truly the order of bony repair, regenesis of the craniofacial skeleton will first require recruitment of provasculogenic and angiogenic factors to ensure an adequate blood supply before osteogenesis.

Stem Cells

Stem cells have the powerful osteogenic potential given their ability to differentiate into osteoblasts. There are numerous techniques to stimulate stem cell-driven osteogenesis. These include direct implantation of undifferentiated cells, implantation after in vitro differentiation, or stimulation of native stem cell differentiation via the introduction of cytokines. Given the challenges of stem cell transplantation in the clinical setting, future work will require a focus on methods for optimizing stem cell harvest and scaffold-based delivery.[10]

Stem cells can be used in a variety of ways to supplement osteogenesis. They can be implanted into living tissue and allowed to differentiate into the surrounding tissue type. They can be implanted after they have differentiated in vitro. Finally, endogenous cells can be stimulated via administration of specific cytokines or growth factors, such as VEGF and bone morphogenic protein.[6]

Stem cells can be classified by their plasticity. They are categorized as totipotent, pluripotent, multipotent, and progenitor. Totipotent and pluripotent stem cells retain the broadest capabilities of differentiation. Totipotent cells can differentiate into any cell type whereas pluripotent cells can differentiate into any cell with the exceptions of totipotent

stem cells and placental cells. Multipotent cells differentiate into cell types specific to the tissue in which they are found. Progenitor cells are the least plastic and represent the most common type of stem cell in the adult body.[6]

Alternatively, stem cells can be categorized by their source: embryonic, fetal, or adult. Embryonic stem cells retain the greatest plasticity, followed by fetal stem cells. Umbilical cord mesenchymal stem cells may be used in place of embryonic stem cells, which are more difficult to harvest.[11] ASCs, found both in tissue and circulation, are the least plastic yet retain the ability to further differentiate into a great number of cell types. For instance, adult bone marrow stem cells can differentiate into cells as diverse as cardiac myocytes, neurons, and hepatocytes.[6]

Mesenchymal stem cells derived from adult bone marrow are potentially useful for craniofacial tissue engineering of bone, adipose, muscle, and cartilage.[10] Kaigler et al found that implanted tissue repair cells led to an increase in alveolar bone regeneration and decreased need for secondary bone grafting as compared with conventional guided bone regeneration.[12] Adult bone marrow stem cells represent only 1 per 100,000 bone marrow cells. Circulating ASCs are only 0.01% of cells in circulation.[6,10] To have a therapeutic effect on reconstruction, a high concentration of stem cells is needed at the site of interest.[10]

Adipose-derived stem cells (ADSCs) may also be used extensively in osteogenesis. Similar to bone marrow stem cells, they are mesenchymally derived and have a supportive stroma for cell differentiation. Yet opposed to bone marrow-derived stem cells, larger quantities may be harvested with less pain.[13] Zuk et al engineered a lineage of a population of stem cells derived from human lipoaspirates. They demonstrated that these cells are capable of differentiating into multiple different types of cells, including osteogenic cells through their expression of osteogenic-specific genes.[14] Yang et al demonstrated the viability of ADSCs osteogenic capabilities by engineering biomimetic scaffolds cross-linked with rabbit ADSCs along with collagen into critical-sized defects in rabbit radii. Complete repair of the defect was achieved in 12 weeks, suggesting a role for the use of ADSCs in osteogenesis.[13]

Bone Morphogenic Protein and Vascular Endothelial Growth Factor

Various proteins may be used to stimulate osteogenesis and neovascularization. Bone BMP is part of the transforming growth factor β subfamily and has been successfully used to promote new bone growth. VEGF has been used in conjunction with a BMP to enhance bone formation by stimulating angiogenesis. Further research is needed to elucidate both the optimal concentration for bone growth and the ideal mode of growth factor delivery.

There are 15 proteins that belong to the BMP family. The various subclasses of BMP bind to mesenchymal stem cell receptor sites and, through signal transduction via Smad proteins, stimulate gene transcription that can stimulate stem cells to differentiate into chondrocytes and osteoblasts which lead to bone formation integral to bone healing. BMP can be found throughout the body, including the perichondrium of the craniofacial skeleton.[4] BMP-2, BMP-6, and BMP-9 have been demonstrated to be the most effective osteoinductive BMPs.[15–17] Their efficacy in osteogenesis depends on concentration, frequency of dosage, carrier type, and site of implantation.[18]

Much of the work done exploring the roles of BMPs in bone regeneration has been done using calvarial defects.

Moghadam et al successfully used a combined BMP-rich polymer-based gel with BMP-impregnated allogeneic bone graft in a patient with a large mandible defect unsuitable for free flap reconstruction given a history of extensive total body radiation. The defect spanned from sigmoid notch to just distal to the ipsilateral first premolar. This patient showed both radiological and histological evidence of new, healthy bone formation with good functional results at 9 months.[4]

Ferretti et al compared autologous bone grafting to a synthetic osteogenic device in the reconstruction of 13 patients with segmental mandibular defects following surgical ablation of benign tumor or trauma. The synthetic construct consisted of allogeneic bone matrix impregnated with partially purified bovine BMP placed onto a titanium mesh, which was used to span the defect. Only two of the six patients who received the synthetic construct showed histologic evidence of bone induction. The authors sited poor angiogenic response as the primary reason for failed osteogenesis.[19]

Takahashi et al demonstrated that biodegradable gels impregnated with BMP-2 could be used to regenerate skull bone defects in cynomolgus monkeys. Similarly, BMP-7 was used to regenerate critical-sized calvarial defects in a pig model, suggesting a use for BMP-7 in the regeneration of pediatric craniofacial defects.[14] Commercially available BMP-2 has proven to be effective in regeneration of craniofacial defects in Apert and Crouzon syndromes. In this study, lyophilized cartilage strips interspersed with BMP were used to promote craniosynostosis with interval computed tomography scans demonstrating early evidence of calcification.[20]

Peng et al showed that delivering stem cells engineered to produce VEGF and BMP-2 enhances bone formation by stimulating new local angiogenesis. Further, hypoxia and VEGF have been linked to higher levels of BMP-2 in microvascular endothelial cells.[6]

VEGF interacts differently with various classes of BMP in osteogenesis. Previously, VEGF has been linked to BMP-6 via an internal ribosome entry site and subsequently transformed into recipient stem cells.[21] The stem cells were mounted on a polylactide/polyglycolide (PLAGA) construct and grown in an in vivo setting. The animals with the linked VEGF and BMP-6 implants showed higher alkaline phosphatase (ALP) activity, vessel growth, and more pronounced mineralization as compared with VEGF and BMP-6 alone. ALP levels were 2.4 times higher in cells transfected with VEGF and BMP-6 and 1.3 times higher in cells transfected with BMP-6 or VEGF alone as compared with the control group containing only PLAGA.[21] After 2 weeks, cells transfected with

both VEGF and BMP-6 showed a greater bone volume density as compared with the BMP-6 group alone and in the VEGF group alone, respectively. Similarly, there was nearly a three-fold increase in the number of blood vessels in cells transfected with both growth factors or with VEGF alone as compared with the remaining groups (150 blood vessels per scaffold as compared with almost 50 in the group transfected with BMP-6).[21] These results demonstrate that VEGF enhances angiogenesis in vivo while VEGF and BMP-6 additively enhance osteogenesis.

Growth Factor Delivery

Protein-based, gene-based, and cell-based techniques have been developed to deliver stem cells and proteins to local tissue. These techniques involve implantation of growth factors, cells or genes onto biosynthetic scaffolds. In the future, it will be necessary to elucidate the optimal carrier and growth factor to stimulate bone healing.

There are several strategies of inducing stem cells and local tissue to engage in bone formation and angiogenesis. Broadly, these can be divided into protein-based, gene-based, and cell-based techniques.

Proteins such as exogenous growth factors and cytokines can be seeded or cross-linked into biosynthetic constructs and implanted into the areas of interest. Although altering the chemical properties of the synthetic material stands the chance of weakening the construct, this strategy has been effective in the animal model for repair of craniofacial defects including the mandible, zygoma, and calvarial bone.[10] Further trials are needed to further elucidate the efficacy and drawbacks of synthetic constructs cross-linked with growth factors.

Gene-based strategies can be divided into modes of transmission: viral or nonviral. The adenovirus is a common vector and has been described as a vector for VEGF and BMP-2 in dorsal nasal bone defects in mice.[10,22] Adenovirus is advantageous in situations requiring short-term repair and highly targeted delivery over several weeks.[23] Adeno-associated virus was capable of inducing expression of constitutively active receptor such as kinase-2, BMP/VEGF, and receptor activator of nuclear factor kappa-B ligand in rodent models.[23] Nonviral methods, including introduction of genes via conjugation or in solution, have been limited by low in vivo gene transfer success rates.

Cell-based techniques include implantation of stem cells onto biocompatible scaffolds, which provide a three-dimensional structure within which these cells may proliferate.[24] These scaffolds can then be implanted into defects where the stem cells have the potential to differentiate into the local tissue type.[6] The ideal synthetic bioimplant serves as a medium for interaction of stem cells with growth factors and signaling proteins.[4] It should have several characteristics, including chemical inertia, mechanical strength capable of supporting load-bearing areas, ease with molding and contouring to the recipient site, absorbable and replaceable by native living tissue, able to undergo an optimal rate of degradation, as well as be noncarcinogenic.[25] It should also have good porosity and ideal geometry. Established techniques of scaffold fabrication include particulate leaching, phase separation and inversion, porogen methods, spin casting, and electrospinning.[26] Solid free form fabrication techniques include three-dimensional printing, fused deposition, stereolithography, and robocasting.[26]

Synthetic Scaffolds

The process of new bone formation requires the combined mechanisms of osteoconduction, osteogenesis, and osteoinduction. A successful synthetic scaffold will need to mimic these processes to repair bony defects. Materials used for these scaffolds have included hydroxyapatite (HA), calcium carbonate, poly(propylene fumarate) (PPF), and PLAGA constructs. Current research has been centered on further defining these synthetic scaffolds include optimizing their absorptive and structural properties.

Osteogenesis broadly refers to the formation of new bone. Osteoconduction is the process whereby new bone grows into a distant site, graft or implant. Osteoinduction is the process by which osteogenesis is induced, often by chemical means and cell-to-cell communication. An example of osteoinduction is the stimulation of mesenchymal stem cells native to a regeneration site to differentiate into bone forming cells.[4] All three processes are important steps in healing bony defects using synthetic implants.

Autogenous bone grafts are commonly used for repair of mandibular defects. They carry the benefit of good osseointegration (bonding of autogenous material to the surface of bone without formation of a fibrous layer in between) and osteogenesis.[25] However, they carry the disadvantage of donor site morbidity, poor bone volume, and the risk of graft failure. Causes of morbidity may include excess blood loss, neurologic deficits, and chronic donor site pain.[18] Allogeneic bone grafts lack these disadvantages and provide good osteoconduction as well as osteoinduction if prepared appropriately.[4] There is the possibility of transmitting disease via allogenic grafts; however, stringent screening procedures reduce these risks.[4]

"Bioactivity" refers to an implant's capability of osteoconduction as well as osseointegration.[25] Most synthetic implants are composed of calcium or aluminum. Calcium phosphate apatite compounds, including HA, are useful because of their capability of osseoconduction and osseointegration. HA comes in two basic forms: ceramic and nonceramic. The benefit of a nonceramic or "cement" HA is that there is no loss of volume of the implant overtime.[25] HA cement has been successfully used for repair of large cranial defects with good results. For instance, in the rat model, the addition of HA to collagen has been shown to improve stiffness and interconnectivity after implantation in a critical-sized rat calvarial defect.[27]

Unlike HA, calcium carbonate implants have the capability to resorb. These implants are osteoconductive, but, unlike HA, calcium carbonate will be resorbed by osteoclasts and bone will be laid down by osteoblasts in its place. However, calcium carbonate is susceptible to fracture after implantation. The

main clinical use has been in repair of burr holes in neurosurgical cases. Future possible uses of calcium carbonate include repair of pediatric craniofacial defects, as bone replacement would be beneficial in a population with such high rates of remodeling and growth.[25] Similarly, calcium phosphate (CPC) may be useful due to its high bioconductivity. In the first study of its kind to investigate the addition of collagen to CPCs, Thein-Han et al showed increased numbers of human umbilical cord stem cells on all CPC-containing scaffolds.[11] Those scaffolds seeded with collagen also showed enhanced cell attachment, osteogenic differentiation (as evidenced by increased levels of ALP, collagen I, and *Runx2* gene expression), mineralization, and extracellular matrix development as compared with those without collagen. In this study, the implants were injectable, allowing for ease of use in repair of irregular defects.[11]

However, like calcium carbonate, calcium phosphate is prone to fracture. This problem may be corrected by the addition of a synthetic polymer mesh such as chitosan to the scaffold, which provides mechanical support in addition to a substrate for cell proliferation. Weir et al examined the effect of chitosan-incorporated scaffolds on human mesenchymal stem cells and found an increase in flexural strength due to a reduction in the porosity of the scaffold.[28]

PPF, an unsaturated, linear polyester macromer has been shown to be osteoconductive and biodegradable. Henslee et al examined the mechanical properties of cement-containing unsaturated PPF and cross-linked PPF microparticles.[29] Mechanical testing demonstrated that adding cross-linked microparticles significantly increased the compressive modulus and the compressive strength of the cement in addition to reducing the temperature increase on cross-linking. The mechanical stability of these constructs is consistent with a prior clinical study by Bruens et al that displayed the minimum requirements for maintaining structural strength, thereby illustrating a potential for future use of this bone cement in the repair of craniofacial fractures.[30]

Polyamide (PA) has been shown to possess good biocompatibility with organic human collagen and exhibit enhanced mechanical properties. Given its mechanical strength, PA has been used in combination with HA to compensate for HA's brittleness and tendency for fatigue. Li et al used a synthetic biomimetic PA/HA scaffold to investigate the osteogenic potential of BMP-7 transduced mesenchymal stem cells. They used immunohistochemical staining with ALP and collagen I to verify bone growth and measured a greater degree of staining in addition to greater bone density in those cells transfected with BMP-7 relative to controls.[15]

PLAGA copolymer constructs have proven to be excellent scaffolds for tissue engineering due to their improved strength and absorptive characteristics over HA.[10] As mentioned earlier, they have been used in our laboratory as a scaffold for cellular ingrowth for osteogenesis in the subocutaneous environment.[31] However, PLAGA constructs carry certain disadvantages, including variable strength and time to absorb as various additives will alter their innate properties.[10]

Tissue-guided regeneration with both resorbable and nonresorbable polymers has been evaluated with mixed results. In some instances, the use of membranes has resulted in an increase in bone volume by approximately 90% over a period of 6 to 8 months.[32] In others it has been shown that the treatment with bioresorbable membranes such as HA/β-tricalcium phosphate and bovine-derived xenograft do not produce as much improvement as the use of autogenous spongiosa does. This outcome was measured as late as 12 months after the treatment.[33] Nevertheless, tissue-guided regeneration has been shown to have a much more positive impact on clinical attachment and probing depth reduction in the treatment of intrabony and furcation defects as compared with open flap debridement.[34]

Difficulties associated with bone healing after pre- or postoperative radiation on vascularized bone grafts is well documented in certain animal models.[35] However, a randomized study on autologous and allogeneic grafts has indicated that the failure rate associated with irradiated grafts is not significantly higher than that of the controls.[36] Such discrepancies in reported result can be attributed to numerous factors. Different animals have different rates of bone regeneration, in vivo experiments that simulate human conditions are difficult to conduct as fractionated schedule need repeated anesthesia, the experimental setup (radiation type, radiation dose, targeted tissue) differ across studies.[37–41] Thus, the development of an animal model to effectively evaluate the impact of radiation and drugs to counter those effects is critical to discern further understanding of the process.

Distraction Osteogenesis

Distraction osteoneogenesis (DO) is a clinical example of bone regeneration that capitalizes on intrinsic neovascularization and osteogenesis after a surgical osteotomy creates two vascularized bone surfaces.[42]

DO was first described in 1905 for the surgical treatment of limb length discrepancies, but did not gain widespread use in orthopedics or maxillofacial surgery until the later part of the century.[43–45] DO is used in maxillofacial surgery primarily to lengthen mandibles and the orbital suprastructure in patients with growth abnormalities. While its clinical use is limited, DO does not require the introduction of exogenous growth factors, scaffolds, or stem cells, and serves as an example of the interplay of the endogenous growth factors, stem cells and neovascularization discussed above.

DO is divided into three phases: latency, distraction, and consolidation. The latency phase begins immediately after the creation of the osteotomy and stops at the beginning of active distraction. During latency, the same growth factors are seen as in the early stages of fracture repair (e.g., IL-1, IL-6, BMP-2, BMP-4, VEGF C).[44] At the onset of the active distraction phase, the primary inflammatory processes have been completed. Traction is then placed on the fracture callous at a specified rate. As the callous is stretched, a zone rich in chondrocyte-like cells, fibroblasts, and oval cells forms called the fibrous interzone.[46] This area is associated with differentiating osteoblasts that deposit osteoid along collagen bundles. This osteoid/collagen zone is referred to as the "zone of microcolumn formation" (MCF). In between the MCF and

fibrous interzone, there is an area of rapidly proliferating cells known as the "primary matrix" or "mineralization front" (PMF). Once the distraction phase stops, it allows for consolidation phase to begin. In this phase, the osteoid undergoes mineralization and subsequent remodeling.

The molecular mechanisms underlying the osteogenic change seen during DO are not fully understood. It is thought that living tissues become metabolically activated by slow, constant traction in a process known as mechanotransduction.[44] In the early latency phase, BMP-2 and BMP-4 rise, likely to direct the precursor cells into chondrogenic/osteogenic cells.[42] After distraction has stopped, BMP-2 and BMP-4 disappear.[47] Application of exogenous BMP-2 has been shown to shorten the treatment time of distraction and accelerate bone formation during the consolidation phase.[43] Tumor growth factor (TGF)-B is detected near the end of the latency phase, and is detectable throughout the distraction gap during the distraction phase. It is thought that TGF-B suppresses osteoblast maturation until the consolidation phase.[48] In addition to change in growth factor levels, DO increases demand on surrounding tissues to provide more blood flow. VEGF-A is thought to be the primary inducer of neoangiogenesis during DO, and its expression is localized to osteoclasts at the MCF and maturing osteoblasts at the PMF.[49]

Manipulation of the DO process has been fruitful clinically, and yielded greater understanding of the processes of osteogenesis and neovascularization. The intermittent application of parathyroid hormone (PTH) has been shown to have an anabolic effect on osteogenesis, increasing measures of mineralization and accelerating fracture consolidation.[50] The effect of PTH on angiogenesis remains unknown, though recent reports have shown intermittent PTH application to reverse radiation-induced hypovascularity in DO bone.[51] On the neovascularization side of the DO process, exogenous application of deferoxamine has shown to quantifiably increase vascular response in the DO process and decrease the time required for the consolidation phase.[52] Application of ADSCs in combination with BMP-2 has been shown to accelerate rapid DO[53] and application of mesenchymal stem cells produce a broad array of growth factors that enhance the DO process.[54]

Additional Considerations

While having the appropriate scaffold, cell type, and blood supply is important for new bone growth, the stimulated cells must also receive the appropriate signals to grow in a regulated, organized fashion. These signals include mechanical, chemical, and even electrical stimuli. These signals must then be communicated between cells for the coordinated response needed to guide osteogenesis. Perhaps we can engineer better bone substitutes as we learn more about the physiology of bone healing and intercellular communication.

Wolff law states that bone is resorbed and deposited in areas of greatest stress. Clinically, this is demonstrated by the resorption of bone when it is underused or the opposite with exercise induced stress to the bone. Within the mandible, we know that physiologic stress stimulates bone growth along trajectories of the applied force.[55] Recent studies have implicated the osteocyte as the cell that is able to detect mechanical stresses and communicate shear forces with other cells via canaliculi.[56,57] Osteocytes make up over 90% of the cellular composition of bone. The cell body within the lacuna sends out dendritic processes into canaliculi that serve to connect the cells through the mineralized matrix. Both in vivo and in vitro studies have suggested that the interstitial fluid within bone may be important in the transduction of pressure that leads to osteogenesis.[56,58] How the mechanical stress is converted to an understandable signal at the cellular level is not entirely understood but may involve biochemical and even electrical responses.

Recent studies have shown that osteocytes are capable of producing proteins important for osteogenesis and mineralization including dentin matrix protein 1, phosphate-regulating neutral endopeptidase on chromosome X, and matrix extracellular phosphoglycoprotein (MEPE).[59] In a rat tibial model, applied stress led to upregulated expression of MEPE after just 6 hours. This protein is produced by osteocytes in humans and plays a key role in bone remodeling, bone mineralization, and even dentin mineralization.[60] MEPE may be important for osteogenesis across synthetic constructs. MEPE has proven to stimulate osteoblastic activity in the in vitro setting.[61] When bonded to HA scaffolds and implanted into calvarial defects, MEPE is able to increase bone area by nearly 10-fold.[61]

Piezoelectricity refers to the electric charge generated within an object as a result of applied mechanical pressure. This is a reversible process as mechanical force can be produced via electrical stimulation of a material that has piezoelectric properties. Early studies proved the ability to resorb or grow bone based on bone polarity and applied current. Within bone, collagen is the piezoelectric component.[62]

Electrical signaling within tissue is one means of intercellular communication during wound healing. When tissue is injured, ions flux across the damaged cellular membranes and generate a local direct current. This local current may be important for directing wound healing as inflammatory mediators, growth factors, and reparative cells are drawn to the site of injury to begin the healing process. Cellular activation and the location of secreted extracellular matrix by osteoblasts can be altered by the application of an external current, implicating the importance of electric charge across the cell membrane.[63,64] It has been established that tissues that are able to generate their own electric charges are better able to regenerate.[65] With the application of an external electrical force, cellular organization and realignment can be stimulated in osteoblasts which have been proven to play a role in osteoinduction and osteogeneis.[66,67] Surgically implanted electrodes have been used clinically with some success for long bone fracture healing in cases of nonunions as well as total joint replacement surgery.[65] Combined with mechanical stress, electrical fields may help organize intercellular communication and resulting organized osteogenesis across biologic scaffolds used in boney defects.

Cell-to-cell communication occurs via mechanical and chemical signaling via gap junctions. Connexin 43 (Cx43) is a cell surface gap-junction forming protein found on osteocytes. Shear stress in long bones causes Cx43 channels to open and release prostaglandin E2 which is known to be important for bone remodeling and osteogenesis related to mechanical stress.[68] Similarly, mechanical stress to teeth upregulates Cx43 expression in alveolar bone.[69] Biomaterials can be engineered to stimulate gap junction signaling and subsequent osteogenesis. Transfecting bone marrow-derived stem cells with Cx43 lentivirus stimulates increased production of both osteocalcin and ALP in an in vitro setting and increased osteogenesis in an in vivo setting.[70] As bone forms across biological scaffolds seeded with cells, the ability to form organized bone in response to stress is likely closely linked to intercellular communication via proteins such as Cx43.[71]

Conclusion

Further research is needed in several aspects of the field of implantable osteogenic constructs for the craniofacial skeleton. These include finding the ideal biomaterial, exploring the efficacies of protein versus gene-based strategies of osseoimplants, and defining the optimal use of stem cells in repairing craniofacial defects. While small series and case reports exist in humans regarding the use of bioimplants for mandible defects following surgical resection, little exists on midface and mandible fracture repairs.

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