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Bone induction in craniofacial defects[1]

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Abstract

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Reconstruction of craniofacial bony deficiencies, whether acquired through trauma or as a result of treatment for disease, is a chronic problem. Although numerous approaches utilizing a wide array of materials ranging from alloplastic materials to autogenous bone grafts have been employed to achieve bony replacement, no ideal clinical approach exists. In this brief review, we will provide an overview of current approaches to treating craniofacial bony defects. We will then discuss advances being made in the design of scaffolding materials and potential candidate cell types with which to design tissue-engineered constructs for craniofacial skeletal repair.

Key words: craniofacial defects; tissue engineering and mesenchymal cells

Introduction

Surgeons who reconstruct the craniofacial skeleton are faced with two broad categories of clinical problems: too much bone or not enough bone. The problems of too much bone occur less frequently and include conditions such as craniosynostosis and temporomandibular joint ankylosis. Although conditions involving excess bone are generally not rectified by applying skeletal tissue engineering strategies, the consequences of surgical procedures to correct bony excess may in turn lead to skeletal deficiencies. In either event, the larger clinical problem of craniofacial bony deficiencies will be the subject of this brief review. Defects or deficiencies of the craniofacial skeleton are quite common, and may be due to congenital or acquired causes. Common etiologies include post-surgical defects following tumor resection or ablation, trauma, and a large number of congenital anomalies including syndromic and non-syndromic craniosynostosis, craniofacial clefts, hemifacial microsomia, and Treacher–Collins syndrome.

Reconstructing craniofacial bony defects presents a surgical challenge whose requirements vary by region. For example, the mechanical loads and motion demands on the mandible are substantially different than those on the neurocranium. Despite these variable requirements, the gold standard for craniofacial reconstruction is autogenous bone usually harvested from the skull or ribs. While these sources produce excellent results, autogenous bone grafts are subject to unpredictable resorption, infection, donor site morbidity, and limited sources. As a result of these potential problems, alternative strategies such as cadaveric bone and non-autogenous materials have been investigated and used clinically. Not surprisingly, these strategies for bone replacement also have associated problems such as potential disease transmission, infection, loosening, etc.

Given the donor site limitations in terms of quantity of donor bone and complications of non-autogenous materials such as metals and plastics, alternative strategies for bone induction to repair craniofacial defects are being actively pursued. Current research regarding the healing of calvarial defects will be briefly reviewed.

An enduring problem

Perhaps one of mankind's oldest medical problems has been what to do with a defect in an otherwise normal skull. Evidence of trephination can be found in skulls dating back to nearly 10 000 BC, although the oldest craniofacial surgery with a known indication (intracranial infection) was performed *c.* 4500 BC (1). Although the indication for trephination may have changed over the past 6500 years, from the performing of mystical rituals and the release of evil spirits, to the evacuation of a hematoma or the correction of a skull deformity, surgeons of today find themselves contemplating the same puzzle that confronted their historical colleagues. Namely, 'What on earth do I use to fix that hole?' Although it is unclear with what frequency the craniums of patients from older civilizations were opened, it is abundantly clear that today we are performing more procedures on the craniofacial skeleton than ever before. These operations run the gamut from the repair of simple defects incurred from trauma to complex, three-dimensional reconstructions of dysmorphic craniofacial features secondary to congenital defects. The cost of such procedures is not trivial, with estimates from the United States Healthcare Cost and Utilization Project suggesting that over $250 million dollars was spent in 2001 alone on surgical procedures in children under the age of 17 (2). Although within the Plastic and Reconstructive Surgery community craniofacial surgery is primarily a pediatric specialty, the contribution of procedures on the adult craniofacial skeleton to healthcare expenditures is equally germane, with the cost to repair facial fractures alone approaching $400 million dollars (2). This figure continues to rise if one includes other procedures such as craniectomies or reconstructions following tumor resection. In fact, it is arguably in the adult and elderly population that the greatest need for an effective regimen with which to treat calvarial and bony deficiencies exists. This is the patient demographic that does not possess the innate osseous regenerative potential that children do, and may have general compromise of skeletal healing because of other factors such as osteoporosis, radiation damage, and malnutrition.

Standard approaches to craniofacial reconstruction

Despite centuries of human experience with this problem, the tremendous variety of resources that can be employed to effect craniofacial reconstruction or calvarial defect repair demonstrates the lack of a highly efficacious, complication-free treatment strategy. Materials employed run the gamut from alloplastic substances including metal, glass, and plastics (such as polymethylmethacrylate) to demineralized bone matrix to autogenous bone (3,4).

Although alloplastic substances may be cheap, readily available, and easily contoured to conform to various defects, they are inert, non-biodegradable substances that offer little if any ability to osseointegrate with host bone (3). Although some degree of osseointegration may be facilitated by manipulating the physical properties of these materials, such as creating porous polymethylmethacrylate constructs, bony ingrowth is limited to the periphery and probably only serves to enhance prosthetic fixation (5). These materials are also permanent, and thus serve as a constant, potential nidus for infection.

Currently, the gold standard for the repair of calvarial defects is bone grafting. Bone grafts can either be in the form of autogenous bone taken from the patient being reconstructed, or allogeneic bone from a cadaveric donor. The grafting material of choice is split-thickness skull calvarial autografts. Other potential choices for cranioplasty include split-rib and iliac crest autografts, although these donor sites are subject to complications such as persistent pain, chest wall deformity, and pneumothorax (4,6). The advantages of using autogenous bone grafts include removing the risk of rejection, a high degree of osseointegration, and immediate rigid coverage of the defect. However, autografts are hindered by a limited supply (especially in children under 6), and both auto- and allografts are subject to significant resorption (7,8). With the exception of cadaveric allografts, there exists the possibility of donor site morbidity including pain, infection, and irregular calvarial donor site contour and appearance. The use of cadaveric allografts may be complicated by disease transmission and graft vs. host disease. Bone grafts are not malleable, and thus the ability to achieve appropriate contouring of the transplanted tissue is limited, and may therefore lead to less than ideal esthetic outcomes.

Another regularly utilized material derived from autogenous or allogeneic bone is demineralized bone matrix. Demineralized bone matrix, first described by Urist in 1965, is an acellular, devitalized derivative of native bone that maintains osteoconductive and osteoinductive properties (9). The ability of demineralized bone matrix, now available in a multitude of formulations, to induce ectopic bone formation is attributable primarily to the presence of bone morphogenetic protein (BMP), a powerful osteogenic growth factor of which BMP2 is perhaps the best characterized (10). Demineralized bone matrix has been used extensively in the human craniofacial skeleton, and has several advantages over unprocessed bone graft including ease of handling, elimination of donor site complications, and decreased immunogenicity (7). One main drawback is the pronounced variability in the osteoconductive and osteoinductive properties of demineralized bone matrix from batch to batch (11). Furthermore, demineralized bone matrix is also subject to complications such as incomplete integration, infection, and loosening.

Thus, although the techniques that we have on hand and are currently employing to repair craniofacial defects are adequate, the need for an optimized treatment strategy still exists. Theoretically, an ideal construct with which to reconstruct the craniofacial skeleton would be possessed of several characteristics. It would be non-immunogenic, non-pyrogenic, and bioresorpable, so as to leave no trace of its existence over time. It would be biologically active, preferably osteogenic, in order to induce bone formation within the construct itself and from the surrounding host tissue. An ideal construct should be easily obtainable in, or lend itself to the creation of, recipient-specific shapes to facilitate its application in the complex topography of the craniofacial skeleton. Furthermore, it should be strong enough to bear a mechanical load without failure. Research into the design and fabrication of such a construct for skeletal tissue engineering can globally be divided into the two likely components of the construct: 1) the supportive osteoconductive/osteoinductive framework, or scaffold, and 2) the osteogenic cellular building blocks with which it will be seeded.

Scaffolding materials

The foundation of any construct for craniofacial skeletal reconstruction is the scaffold itself, the backbone upon which bone is formed. A scaffold material must adequately reproduce the physical and chemical properties of natural bone in order to promote the attachment, proliferation, and differentiation of both seeded osteoprogenitor cells and surrounding recipient tissues. Additionally, the three-dimensional structure of a scaffold is of critical importance, as proper bone development is dependent on three-dimensional cellular interactions (12). A review of the literature reveals that a nearly overwhelming variety of substances are employed by researchers seeking to generate a scaffolding material that possesses all the desired traits of an ideal scaffold. In order to bring some organization to this potpourri, scaffolding materials can be most easily classified as natural scaffolds, polymer scaffolds, and mineral-based scaffolds.

Natural scaffolds

Natural scaffolds are comprised of materials which occur in nature (although not necessarily in humans), and include materials such as calcium alginate,

hyaluronic acid, collagen, and chitosan. For example, chitosan is derived from the exoskeleton of crustaceans, and is composed of poly *N*-acetyl-D-glucosamine (13). These scaffolding materials are all capable of supporting bone formation to a greater or lesser extent (13–17). Although the natural scaffolds are osteoconductive, and some such as chitosan may be osteoinductive, these materials do not have the structural integrity to withstand significant stress or strain, and therefore are not well suited as vehicles for bony tissue regeneration in areas of the craniofacial skeleton where mechanical loading is a factor. Additional limitations include difficulty in sterilization, as both gamma radiation and heat sterilization techniques can significantly modify the biochemical properties of natural polymers, potentially altering the ability of these biomaterials to effect osseous regeneration (18).

Polymer scaffolds

One of the most heavily researched classes of scaffolds are the polymer scaffolds. Polymer scaffolds are highly crystalline thermoplastics typically composed of any of a number of alpha-hydroxy acids, such as polylactic acid, polyglycolic acid, polydioxanone, and polycaprolactone. Synthetic scaffolds can either be homopolymers, consisting of one of the above acids in repetition, or a copolymer, such as polylactic-co-glycolic acid (PLGA) (19). These materials are extraordinarily strong prior to biologic modification (as evidenced by their routine use in suture material such as Vicryl^{TM}), which makes them a favorable substrate to use in the context of a dynamic mechanical environment. Polymer scaffolds are biodegradable, undergoing hydrolysis in response to local factors such as pH with the end product of degradation being carbon dioxide and water. The strength of the scaffold and rate of absorption can be altered by modifying fabrication variables such as type(s) of polymer, porosity, and crystalline structure, enabling manufacture of scaffolds with levels of biodegradability that can be specified for individual applications (20). Scaffolds composed of synthetic polymers are generally considered to be osteoconductive, allowing ingrowth of bony tissue, but are not osteoinductive in their native form (21,22). However, due to their ability to bind growth factors and deliver them locally in a metered fashion (alpha-hydroxy acid polymers are used as drug delivery vehicles), osteoinductive scaffolds can be designed by incorporating such factors as BMP2, theoretically elevating the utility of these materials as the scaffold of a bony tissue engineering construct (23).

Another class of polymer scaffolds are the hydrogels. Hydrogels are formed by the polymerization and cross-linking of chemicals such as *N*-isopropylacrylamide and acrylic acid (24). These hydrogels have many unique properties, which lend themselves to tissue regeneration strategies. Hydrogels can be produced in such a fashion that they adopt different physical properties depending on their temperature. These thermoresponsive hydrogels can be gelatinous at room temperature, but polymerize at body temperature significantly increasing their rigidity (25). This would theoretically make hydrogels amenable to delivery via minimally invasive approaches, such as injection. Hydrogels can also be modified by the addition of specific peptides during synthesis, markedly altering the properties of the scaffolds. For example, polypeptide cross-linkers can be added to the gel, allowing tissue-specific factors such as matrix metaloproteinases to degrade the construct. Other peptides that impart specific instructions to attached cells can also be provided, such as the inclusion of RGD (Arg–Gly–Asp) peptide motifs to increase osteoblast adhesion and proliferation (26).

Mineral-based scaffolds

Mineral-based scaffolds, or bioceramics, have long been recognized as potent inducers of bone formation (27). This property stems from the fact that bioceramics replicate the calcium phosphate mineral occurring in natural bone, usually in the form of beta-tricalcium phosphate or hydroxyapatite, with hydroxyapatite being both structurally and chemically comparable to the calcium phosphate found in mineralized bone (19). A key feature of bioceramics which makes them extraordinarily attractive as a candidate scaffolding material for skeletal tissue engineering is their bioactivity. Upon implantation into the body, bioceramics undergo a surface modification generating a layer of hydroxylcarbonate apatite, which bonds to host tissue with extraordinary strength (28). By mimicking the endogenous crystalline lattice of bone, mineral-based scaffolds are both osteoconductive and osteoinductive. Recent data suggest that certain bioceramics may also

be osteogenic when seeded with the appropriate mesenchymal progenitor cells, possibly due to their ability to bind local growth factors such as BMPs or by a direct interaction between seeded cells and the specific structural microenvironment created by the scaffold (28–31). While bioceramics may be the most similar scaffold material in structure and composition to mineralized bone, the properties which make them so desirable also hamper their widespread implementation. The inherent porosity of these scaffolds, which facilitates bony ingrowth and thus osseointegration and resorption, makes these constructs brittle, and thus prone to stress fracture when placed in load-bearing situations (32).

Cellular resources

The second major component to a tissue-engineered construct for craniofacial repair may arguably be the most important – the cell. The identification of a plentiful, easily accessible stem or osteoprogenitor cell with which to seed an implantable scaffold has been the source of much investigation. Potential sources for stem cells can be divided into embryonic and postnatal. Although embryonic stem cells hold great promise as a powerful source of tissue for engineering purposes and may someday be the gold standard for numerous therapies, their accessibility and utilization is currently snarled in political and scientific debate (33–35). Another source of cells that may have therapeutic potential similar to that of embryonic stem cells are mesenchymal cells derived from umbilical cord blood, as these cells have been demonstrated to differentiate down the osteogenic lineage (36). However, a far more likely and accessible source of progenitor cells are adult or postnatal multipotent cells, of which the bone marrow-derived stromal cell (BMSC) is the most widely utilized. Although it remains unclear whether the mechanism of tissue regeneration effected by postnatal progenitor cells occurs via cellular fusion and rescue or direct lineage-specific differentiation, adult-derived progenitor cells from several sources have been demonstrated to be capable of differentiating into multiple types of tissue including muscle, cartilage, and bone *in vitro* (37–39).

Until recently, BMSCs were at the center of attention for researchers striving to design osteogenic bone tissue engineering constructs, and there is certainly no doubt that BMSCs are a powerful tool for inducing bone formation (40–45). However, the recent discovery of postnatal progenitor cells with osteogenic potential residing in the stromal fraction of adipose has highlighted some of the potential drawbacks of working with BMSCs (46,47). Adipose-derived mesenchymal cells (AMCs) are much more accessible clinically, are available in greater numbers, and expand more rapidly in culture than do their bone marrow-derived counterparts. The capacity of these cells to differentiate into bone may also be less sensitive to the effects of donor age than BMSCs, a desirable trait given the large demand for general and craniofacial skeletal reconstruction in the elderly (Y. Shi, R.P. Nacamuli, A. Salim, M.T. Longaker, personal communication, 2004) (48–50). Recent experiments suggest that the ability of AMCs to form bone *in vivo* is both robust and comparable to bone induction seen when utilizing BMSCs (51–54).

Putting it all together

It is obvious from the above discussion that researchers have many options when trying to decide which type of scaffold to use as a backbone for bony tissue engineered constructs. Similarly, osteogenic constructs have been created utilizing BMSCs and AMCs, as well as other more conventional cell types (54). The options for construct design increase exponentially if investigators opt to maximize the biologic properties of cell scaffolds by combining various materials. Manipulations such as these attempt to create hybrid scaffolds whose end-function draws on the particular strengths of the components, attempting, for example, to merge materials possessed of increased strength with materials with superior osteoinductive capabilities. These strategies are most certainly effective. By combining calcium phosphate ceramics with chitosan or biodegradable fibers, Xu et al. demonstrated that the new composite scaffold had mechanical properties approaching that of cortical bone, while still supporting cellular attachment and proliferation *in vitro* (55,56). Ramay et al. have achieved similar results by combining beta-tricalcium phosphate scaffolds with hydroxyl-apatite nanofibers (57). While the above manipulations aimed to increase the strength of the relatively brittle ceramic scaffolds, other investigators

have utilized polymer scaffolds as the primary scaffold material. This approach may be advantageous when compared with manipulations using other base scaffolding materials, as the polymer scaffolds are easily synthesized into complex shapes, potentially facilitating the design of constructs for the craniofacial skeleton. Eppley et al. have demonstrated increased osteoinduction of PLGA membranes in a rabbit calvarial defect model by applying calcium phosphate to the membrane prior to implantation (58). Data from that study revealed that bone formation was augmented when defects were treated with calcium phosphate-coated membranes. Murphy et al. have tested the ability of a biomineralized PLGA scaffold to enhance calvarial bone formation in rats, noting significantly increased osteoid formation and mineralization vs. non-mineralized scaffolds (59).

Equally important is the ability of these hybrid scaffolds to accelerate bone formation when seeded with an osteogenic cell type, as this is not necessarily true for all scaffolds (60). Our laboratory has recently demonstrated the ability of AMCs to heal critical-sized calvarial defects in mice (54). Cells were seeded onto accelerated apatite-coated PLGA scaffolds and then immediately placed in parietal bone defects. Accelerated apatite is a hydroxyapatite synthesized using a modified technique which dramatically reduces the time needed to coat a substrate from days to hours, and also increases the osteoinductive properties of the apatite (31,61). Detailed assessment of calvarial healing in this model utilizing micro-computed tomography, nuclear imaging, and histology demonstrated that by 12 weeks 70–90% of the area of the defects were filled with mineralized bone. Donor-cell origin of the regenerate was confirmed by fluorescence *in-situ* hybridization for the Y chromosome. No healing was observed on uncoated PLGA scaffolds. Comparable results were also obtained for several other cell types, including BMSCs, osteoblasts, and for all cell types derived from both juvenile (6-day old) and adult (60-day old) animals.

Although the end result of these considerations is an overwhelming amount of peer-reviewed literature (over 100 publications on bone-inducing scaffolds in 2003 alone), these investigations will prove central to the advancement of scaffold design and, ultimately, the implementation of clinically relevant strategies for craniofacial skeletal repair.

Clinical utility and implications

Despite the large body of preclinical studies demonstrating the ability of numerous materials and cell types to enhance bone formation and hasten healing of calvarial and craniofacial defects, there has yet to be significant translation of this wealth of data to the clinical arena. Although research observations made regarding bone–scaffold interactions have been implemented to improve the osseointegration and fixation of polymethylmethacrylate implants in humans, if one excludes the currently established techniques of repair (bone grafting, hydroxy apatite bone pastes, and alloplastic materials) it rapidly becomes apparent that the clinical options are relatively narrow when compared with the breadth of basic research (5,62,63). An example of a recent introduction of a new product into the craniofacial arena occurred in the mid-1990s, with the arrival of the LactoSorb^{TM} (W, Lorenz Surgical, Jacksonville, FL, USA) absorbable plating system. LactoSorb^{TM} is composed of a PLGA polymer scaffold, and has been utilized extensively to effect rigid fixation and stabilization during craniofacial surgery (64–66). Another recent innovation is InFUSE^{TM} (Medtronic, Inc., Minneapolis, MN, USA) Bone Graft (67). This product consists of recombinant human (rh)-BMP2 delivered on a collagen sponge and is used by orthopedic surgeons to augment bone formation during spinal fusion. An absorbable collagen sponge has also been used to deliver rh-BMP2 to tooth extraction sites and to augment alveolar ridge bone formation in humans (67). However, to our knowledge there are currently no readily available clinical therapies for cranial reconstruction that fully capitalize on the research advances being made with regards to materials science and cellular therapies.

Regenerative medicine stands poised at the threshold of the operating room, ready to cross-over from the laboratory bench and approach the bedside. Indeed, given the myriad of studies in various animal models and the difficulty of accurately comparing results between reports, in the end it will most likely be clinical studies that elucidate what the ideal combination of scaffolding material and cellular building block is to effect craniofacial skeletal repair. Until then, the same question will run through our minds as it did through our forebears thousands of years ago; 'What *will* I put in that hole?'

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