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Tissue Engineering Craniofacial Defects With Adult Stem Cells? Are We Ready Yet?

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ABSTRACT: Over three-quarters of all craniofacial defects observed in the US per year are cleft palates. Usually involving significant bony defects in both the hard palate and alveolar process of the maxilla, repair of these defects is typically performed surgically using autologous bone grafts taken from appropriate sites (*i.e.*, iliac crest). However, surgical intervention is not without its complications. As such, the reconstructive surgeon has turned to the scientist and engineer for help. In this review, the application of the field of tissue engineering to craniofacial defects (*e.g.*, cleft palates) is discussed. Specifically the use of adult stem cells, such as mesenchymal stem cells from bone marrow and Adipose-derived Stem Cells (ASCs) in combination with currently available biomaterials is presented in the context of healing craniofacial defects like the cleft palate. Finally, future directions with regards to the use of ASCs in craniofacial repair are discussed, including possible scaffold-driven and gene-driven approaches. **(*Pediatr Res* 63: 478–486, 2008)**

At 75% of all birth defects recorded in the US per year, craniofacial defects, such as cleft palate, are the most common birth defect affecting nearly 225,000 children each year (1). Correction of many of these defects requires extensive surgical intervention using bone-grafting techniques and will often involve numerous procedures over the course of at least a decade. To the child, bone grafting requires extensive healing time—at both the correction site and donor site, runs the risk of infection, results in significant amounts of pain, and does not guarantee complete correction of the defect if the graft fails to integrate within the surgical site. Beyond the child, surgical correction of such craniofacial defects also places a huge emotional and financial burden on the child's family and ultimately places a financial burden on the US health care system. In 2001, data from the US Health Cost and Utilization Project reported that 12,700 cranial bone grafts were performed to repair craniofacial defects in children at a cost of over $549 million (2). As such, there has been a call for alternate approaches that, at a minimum, can decrease the severity of the many side effects associated with surgery. Although the complete elimination of surgery is unrealistic, the creation of cutting edge technologies may decrease the number of procedures and ultimately improve the final outcome of the necessary surgeries. One such cutting-edge technology may be the stem cell.

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THE CLEFT PALATE

Of all the possible craniofacial defects observed in newborns, perhaps the most well-known defect is the cleft palate. Occurring with a frequency of approximately 1 in every 700 per year in the US, the incidence of cleft palate equates to 475 cleft palates per month or 15 clefts per day (3). The layman's term "cleft palate" is actually a combination of soft and hard tissue defects involving the lip and maxilla. Although clefting of the lip only can occur (*i.e.*, cleft lip), it is most often accompanied by a cleft within the palate (*i.e.*, cleft lip with palate). With respect to the maxilla, two main regions are often involved in the cleft palate: 1) the primary palate—the region of the palate anterior to the incisive foramen and 2) the secondary palate—the region of the palate posterior to this foramen. Clefts in the primary palate involving the alveolar process and the lip are referred to as primary palate clefts, whereas clefting in the secondary palate involving just the hard and/or soft palate are termed secondary clefts. More commonly, clefts involve both palates and are referred to as complete cleft palates. These possible clefts can form on one side of the facial midline—unilateral clefts—or can form on both sides—bilateral clefts.

Embryologically, development of the face begins at the fourth week with the migration of neural crest cells toward the head region and their combination with core mesoderm and epithelial cells to become the facial primordia. Within these primordia, mesenchymal tissue derived from neural crest cells will become the facial skeleton with the mesenchymal cells derived from the mesoderm forming the facial musculature (4,5). At 24 d, the primitive mouth forms (stomatodeum) along with the mandibular arch or first pharyngeal arch. Just 2 days later, this stomatodeum is surrounded by five primordia comprised of an unpaired frontonasal prominence, a pair of maxillary processes and a pair of mandibular processes. At 32 d, a thickening of the surface epithelium in the frontonasal prominence produces nasal placodes that become surrounded by the horseshoe-shaped nasal processes (lateral and medial). Growth of the maxillary and medial nasal processes (MNP) pushes the lateral nasal process up and brings the maxillary process and MNP into contact—events that are critical to the

Abbreviations: ASCs, Adipose-derived Stem Cells; **BMP2,** bone morphogenic protein 2; **DMB,** demineralized bone matrix; **ES cell,** embryonic stem cell; **HA,** hydroxyapatite; **MNP,** medial nasal process; **MSC,** Mesenchymal Stem Cell; **PLGA,** poly(lactic-*co*-glycolic) acid; **SVF,** stromo-vascular fraction; **TCP,** tri-calcium phosphate

formation of a continuous upper lip (6). Following contact and fusion, the MNP develops into the intermaxillary segment that will form the central portion of the lip and contributes to palate formation. Outgrowths of this segment into the oral cavity and their fusion forms the anterior or primary palate, whereas the maxillary processes give rise to lateral palatal shelves (7) that fuse to form the posterior or secondary palate. Failure of the intermaxillary outgrowths to fuse results in primary palate clefts with failure of the maxillary processes to fuse producing the secondary cleft. Failure of both of these fusion events results in a unilateral or bilateral complete cleft palate. Because the formation of the lip occurs before development of the palate, it is easy to recognize that most palate defects are accompanied by clefting of the lip. Therefore, the term cleft lip with palate is frequently termed just cleft palate.

Cleft lips and palates have both functional and esthetic implications for children. Functionally, midfacial skeletal growth is affected dramatically by the failure of the facial processes to fuse properly and dramatic alterations to the face, including proper alignment of teeth, can occur. Such malformations can result in social alienation of the child because he or she "looks funny." The development of speech can also be profoundly affected along with increased incidence of ear infections owing to improper draining of the middle ear. In the infant, the cleft can prevent the child from developing normal suction during suckling, thus eliminating the choice of breastfeeding. At the everyday level, the malformed palate necessitates the purchase of specialized bottles that help deliver milk to the back of the throat, where it can be swallowed normally. It becomes easy to see that correction of craniofacial defects like the cleft palate represents a tremendous leap forward in the medical field.

Close to 600,000 bone grafts are performed in the US each year with approximately 6% of these grafts craniofacial in nature (8). To the surgeon, repair of the cleft palate is imperative as it provides bony continuity and stability to the alveolar ridge, allowing for proper tooth movement and eruption, provides support to the lip and nose and closes the oronasal fistula. To the family, repair of the defect dramatically improves the quality of life for the child. The earliest repairs of palates were performed by suturing the palatal halves together but were immobile and dramatically impacted the speech and swallowing of the patient. Repair of the alveolar portion of the cleft has been even more complicated. Although initial closure of the lip and palate can easily be performed within the first 6 mo, most patients will require secondary bone grafts over the next decade to correct the growing alveolar portion of the mandible. Add to this, the need to consider tooth eruption and alignment and the reconstruction of a cleft palate becomes extremely complicated. Current reconstructive techniques combine the advantages of prosthetic, allogeneic and autologous (*i.e.*, bone grafts) materials. In the last 50 y, bone grafting of the alveolar cleft defect using autologous cancellous bone grafts has become the gold standard for reconstruction because of their osteoconductive and osteoinductive properties. However, these properties do not necessarily guarantee success as the graft may not fully integrate into the host bone and may undergo a certain level of resorption. Add to this donor site morbidity, disease transmission and contour irregularities and the craniofacial surgeon faces a significant challenge in the treatment of the cleft palate (9). To solve these problems, the physician has turned to both the scientist and engineer.

CRANIOFACIAL DEFECTS AND TISSUE ENGINEERING—A MATCH MADE IN HEAVEN?

Tissue engineering can be simply defined as the regeneration of new tissues through the combined use of biomaterials and biologic mediators, such as the stem cell. In the field of orthopedics, tissue engineering applications have grown in popularity with numerous studies reporting the healing of long bone and calvarial defects in numerous large and small animal models. However, the application of tissue engineering to craniofacial defects may be more challenging. Simply put, the implanted construct within the craniofacial defect would be under tremendous strain and stress. Numerous studies have examined the magnitude and direction of strain and stress in several long-bone defect models and have begun to develop computer-modeling systems that help the bioengineer understand the mechanical environment within the bone. Unfortunately, understanding the physical environment within the skull does not appear to be as straightforward (for review see (10)). Although not intuitively obvious, the bones of craniofacial arena are under significant amounts of stress—mostly provided by the large muscles of mastication (*e.g.*, masseter). Recent large animal studies have found it difficult to reliably put a "number" to the mechanical loads found within the human skull (11,12), making the design of the "perfect" craniofacial implant for tissue engineering a significant challenge.

CRANIOFACIAL TISSUE ENGINEERING

Pick a Scaffold, Any Scaffold

To the craniofacial reconstructive surgeon, tissue engineering advancements over the last decade has provided a plethora of materials that may be suitable for the healing of craniofacial defects like the cleft palate. At a basic level, tissue engineering scaffolds can be broken down into three groups: autografts, allografts, and xenografts. Today the reconstructive surgeon makes best use of the autograft category, taking bone from another site (*e.g.*, iliac crest, rib) and transplanting it into the cleft defect. With the disadvantages of host-site morbidity and a lack of suitable graft sites and material, the use of xenografts—*i.e.*, bone grafts from animals—would be a good fit. Of course, it is obvious that histocompatability issues between the human patient and the animal donor would preclude its use. In fact, xenografts are not currently allowed in the United States. This leaves the allograft.

As a category, allografts can be organized into two groups: natural and synthetics.

Natural. The natural category is a broad-range category that includes bone powders, chips and fragments. Processed to remove the cellular components, natural materials are osteoconductive but poorly osteoinductive, thus decreasing the robustness of the response *versus* a conventional autograft (13). An alternate natural allograft is demineralized bone

matrix (DMB), the decellularized, organic component of bone. DMB represents a concentrated source of BMPs and has been used in numerous animals systems since its initial description in 1965 (14). Available commercially from tissue banks, the widespread use of DMB in humans still remains restricted as the immunologic properties of donor DMB is unknown.

Synthetic. As an alternative to natural scaffolds, the reconstructive surgeon has available a wide variety of synthetic scaffolds, including ceramics, calcium phosphates and polymers (Table 1). Ceramics [often referred to as hydroxyapatites (HAs)] are a family of calcium phosphate and calcium sulfate materials with a diverse spectrum of mechanical and degradative properties based on their composition and processing (15). Meant to be a substitute for the mineral phase of bone, ceramics are purely osteoconductive but can be combined with stem cells (*i.e.*, marrow) to provide osteogenic potential (15,16) Moreover, degradation *in vivo* can easily be affected by changing parameters such as their calcium to phosphate ratio or their internal surface area (*i.e.*, pore architecture). Similar to ceramics, calcium phosphates are a general term for a large group of scaffold compositions many of which are commercially available today as β-tricalcium phosphate/TCP, or biphasic calcium phosphates (*e.g.*, HA in combination with TCP or HA/TCP). All try to closely match the calcium to phosphate ratio of natural HA and possess excellent bone-bonding ability. Originally used over 20 y ago as coralline HA implants (17), many forms of calcium phosphate scaffolds are used today in orthopedic surgery and have been described extensively elsewhere (18–20) Some calcium phosphates are currently in use clinically repairing cranial defects in the form of calcium phosphate "cements"—a wet paste that can be applied to irregular bony defects and allowed to "cure" thus forming HA (21,22). Such scaffolds would have the advantage of filling the irregular contours of a craniofacial defect. However, the combination of these cements with stem cells has not been fully explored. Finally, many studies now employ the use of organic synthetic scaffolds based on alpha-hydroxy acids (23,24). These scaffolds are usually composed of polyglycolic acid, poly-L-lactic acid or a combination of both (*i.e.*, PLGA) possess limited osteoconductive capacity but when combined with HA technologies become excellent materials for bone repair by stem cells. The last 10 years has seen additions to this large field with the development of scaffolds comprised of a variety of materials including polyvinyl, polycaprolactone, and polyhydroxyalkanoate. Again, too large of a field to adequately review here, numerous excellent reviews detail the use of these organic polymers with and without combination with calcium phosphates and ceramics (25,26).

Table 1. *Synthetic Bone Engineering Composites*

Scaffold type	Commercial name
Chitosan (poly-1,4-D-glucosamine)	
Ceramics	
Hydroxyapatite/HA	
Sintered HA	
Biomimetic HA	
Bioglass	
Calcium phosphates	*e.g.*, Cellplex
β-Tricalcium phosphates	
Biphasic calcium phosphates (e.g. HA/TCP)	
Synthetic polymers	
Poly(lactic-*co*-glycolic) acid	
Poly-L-lactic acid	
CNI-HA	*e.g.*, Healos
Treated metals—titanium, tantalite	
Composites	*e.g.*, Collagraft
CNI/β-TCP, CNI /HA	*e.g.*, Ceraform
PLA/HA/CNI sponges	
PLGA/HA	
Gelatin/chitosan	
PLA/chitosan	

CNI, collagen type I; HA, hydroxyapatite; PLA, poly-L -lactic acid; PLGA, poly(lactic-*co*-glycolic) acid; TCP, tricalcium phosphate.

Adult Stem Cells—The List Keeps Growing and Growing

Today, multiple sources for the isolation of adult stem cells have been identified, including heart tissue (27), umbilical cord blood (28), skeletal muscle (29,30), and the dermis of skin (31). But, to the craniofacial surgeon interested in using tissue engineering, there are two exciting sources of stem cells: bone marrow and adipose tissue.

Mesenchymal stem cells (MSCs). The identification of pluripotent MSCs in the bone marrow stroma over 25 y ago (32) has led researchers to a variety of exciting research avenues. Capable of differentiating to multiple mesodermal lineages, including bone and cartilage, MSCs have become a standard in the field of adult stem cell biology and in regenerative medicine (33–38). So, it is only natural that these stem cells would be used in the repair of significant bony defects caused by trauma, surgery, or disease. Consistent with this, multiple studies have reported the formation of bone tissue both *in vitro* and *in vivo* upon the combination of MSCs and 3D scaffold supports. *In vitro*, a wide spectrum of scaffolds are being combined with MSCs, including, HA/chitosan composites, chitosan or gelatin/TCP constructs, electrospun collagen nanofibers, honeycomb collagen scaffolds, and titanium meshes (39–44). In animals, the scaffolds and model systems used have varied from HA ceramics or HA/TCP constructs for the healing of small bone defects in rodents or larger defects in dogs, rabbits, or sheep (45–49), to complicated biosynthetic composites (50,51), to silk-based biomaterials in the healing of segmental femoral defects in nude mice (52). Each of these studies report encouraging results and espouse the use of bone marrow MSCs in the repair of bony defects.

Adipose-derived stem cells. Historically, the adipose compartment has been considered primarily a metabolic reservoir—effectively packaging, storing, and releasing high-energy substrates in the forms of triglycerides and cholesterol as well as lipid-soluble vitamins. However today, the adipose compartment may be a site for an abundant population of stem cells—the adipose-derived stem cell (ASC) (53,54). Like the bone marrow, adipose tissue contains an extensive cellular stroma comprised of fibroblastic-like cells termed by Rodbell in 1964 as the stromo-vascular fraction or SVF (55). Further work by Hauner expanded this knowledge and postulated that the preadipocytes within the SVF represented a "progenitor"

population, though apparently limited to the adipocytic lineage (56). However, in 2001, Zuk *et al.* showed that the SVF fraction isolated from human lipoaspirates in fact contained cells with multilineage potential and termed these cells processed lipoaspirate cells (53,54). Now renamed ASCs (57), these cells undergo adipogenesis, osteogenesis, chondrogenesis, and myogenesis *in vitro*, suggesting that the SVF fraction of adipose tissue may, in fact, be comprised not just of lineage limited preadipocytes but of multipotent stem cells. Since their initial characterization only 6 y ago, the amount of work performed determining the multipotentiality of the ASC population has been staggering (53,54,58–81). Numerous articles not only continue to document the mesodermal potential of these stem cells but now suggest expanded germ-layer potential by describing their ability to form putative neurons, hepatocytes and pancreatic cells—at least *in vitro* (63,65,66). Today, the researcher interested in ASCs has a wide variety of review articles from which to learn of these cells (57,82–85).

ASCs have also become a hot topic in the world of tissue engineering. Numerous studies have begun to explore the osteogenic potential of ASCs *in vivo* through their combination with a wide variety of scaffolding materials. Groups led by Lee and Hicok were the first to show that s.c. implantation of human ASCs loaded onto HA/TCP or polyglycolic scaffolds could result in the formation of an osteoid-like material (86,87). Subsequent studies have since attempted to confirm this finding in established animal models with limited amounts of success (86,88–90). To improve their ability to form bone, many of these studies treat ASCs with the osteogenic growth factor BMP2. Both Peterson and Dragoo were the first to describe the engineering of well-formed bone by ASCs in athymic rodents with the help of bone morphogenic protein 2 (BMP2) (88,89,91) and several MSC studies have shown that this osteogenic factor can be used in concert with these stem cells also (92–94). Many of these studies claim that increased bone formation can be attributed to the presence of BMP2-treated ASCs. However, the greater majority of these works fail to report levels of healing when empty scaffolds treated with BMP2 were used as controls. Many studies fail to use this construct as a control at all. This omission makes it difficult to determine whether the ASC itself is responsible for the bony healing or if the healing can be attributed to the powerful osteoconductive and osteoinductive effects of BMP2. As such, future studies will be needed to specifically determine the true osteogenic capacity of ASCs without their combination with growth factors.

Craniofacial Engineering Using Stem Cells—The Story So Far

Today's scientific literature seems to detail a litany of exciting studies in which embryonic stem (ES) cells can integrate and heal damaged tissues in both animal and human model systems. Although ES cells are known to form bone, the raging ethical debate surrounding these stem cells will likely make their use in craniofacial procedures all but impossible. As such, the reconstructive surgeon is compelled to look elsewhere for help. The most obvious option has become the MSC from bone marrow. Recently, the use of isolated and expanded MSCs has become more and more popular in the literature. To list a few, MSCs have been combined with HA/TCP scaffolds to help build calvarial and alveolar bone in dogs (95,96), loaded onto gelatin sponges for the repair of calvarial defects in mice (97), combined with polycaprolactone-based scaffolds to repair cranial defects in rabbits (98) and seeded into hyaluronan based polymers for reconstruction of orbital rim defects in pigs (99). Osteo-induced MSCs have also been combined with calcium alginate composites to repair alveolar defects in dogs (100) and cranial defects in sheep (101). Although freeze-dried bone marrow has long been used in the repair of human alveolar clefts (for review see Ref. 102), the use of purified MSCs in cleft repairs is still rare in clinical studies. However, MSCs have recently been combined with platelet-rich plasma to heal an alveolar cleft in a 9-yr-old girl (103). As scaffolds suitable for repairing cleft defects become more available, these studies will surely increase in number.

Although the MSC continues to be viable option for a stem cell population in craniofacial repair, there are drawbacks to the population that must be recognized. Foremost is the pain and stigma associated with the bone marrow harvest. Second is the yield. Although MSCs grow well under standard tissue culture conditions, *ex vivo* amplification is a necessity due to relatively low numbers of MSCs thought to be present in the harvested marrow (1 MSC/10^4–10^6 stromal cells (104)). In light of this, adipose tissue has become an extremely attractive option. In fact, the use of the buccal fat pad in the reconstruction of soft palate, maxillary defects, and palatal clefts has been used for several years in clinical studies with varying results (105,106). Descriptions of purified ASCs in craniofacial engineering appear to be limited in today's literature and their use has also resulted in varying amounts of success. For example, the implantation of osteoinduced rabbit ASCs and gelfoam scaffolds into rabbit calvarial defects did not significantly improve bony healing when compared with controls (107). In contrast, Yoon and colleagues report improved calvarial defect healing upon implantation of PLGA scaffolds seeded with human ASCs maintained *in vitro* in the presence of osteogenic factors before implantation (108). However, the improved bone formation they observed was compared with ASC/PLGA scaffolds maintained *in vitro* in noninductive DMEM. It remains unreported whether acellular scaffolds maintained in osteogenic media were performed as a control or if they produced similar levels of healing as the ASC-seeded scaffolds preinduced toward the osteogenic lineage. Therefore it is difficult to determine whether the healing was due to the activity of the ASC or osteo-inductive factors absorbed to the scaffold. Today, numerous alternatives to conventional HA or polymers are being proposed as supports for the repair of large bony defects using ASCs, including silk-fibrin grafts not unlike those used with MSCs (109), coral matrices (110), or decellularized bone tubes (111).

Yet, despite all this apparent progress, the application of tissue engineering techniques specifically to palatal bone engineering has been limited and progress slow. Repair of palatal defects caused by nonfusion has been performed *in*

vitro using embryonic mesenchyme tissue (112), whereas the insertion of BMP2-coated heparin beads has been found to promote the *in vitro* fusion of small fragments of human palate with its murine host (113). Work in the veterinary field suggests that MSCs may be used to heal soft palate defects in horses (114). Recently, one study by Conejero and coworkers has emerged to suggest that the ASC is being closely examined for its applicability to craniofacial defects, like cleft palate. In this study, rat ASCs were seeded onto conventional poly-L-lactic acid scaffolds and induced in osteogenic medium for 7 d before being inserted into surgically produced cleft defects. The researchers reported significant bone formation in the palates treated with osteogenically differentiated ASCs. However, like the studies of Yoon, which were similar in composition, these authors also fail to report if acellular scaffold controls treated for 1 week in osteogenic medium also healed palates to any degree. Despite this, the application of ASCs to palate defect models and their putative ability to treat these defects is an exciting development.

WHERE DO WE GO FROM HERE?

Several animal models have induced bone formation within long bone and cranial defects by using MSCs treated with or virally-expressing BMP2 (92,94,115–118). Based on the early work of Peterson and Dragoo (88,89,91), it is not unreasonable to think that ASCs, treated with BMP2, would be capable of forming bone within a cleft defect. However, work by Leboy has suggested that BMP2 may not promote osteogenic differentiation of human MSCs (119,120). Similarly, in patients receiving recombinant BMP2 treatment, the regenerative response is several times lower than that previously measured in animal studies (121), suggesting that the response of human cells to BMP2 may not be directly comparable to that observed by animal cells. Although several studies have begun to combine BMP2 and ASCs, surprisingly, to date, no in-depth *in vitro* studies have been performed to confirm if BMP2 can actually promote ASC osteogenesis. Like human MSCs, it is possible that BMP2 has no effect on inducing ASC-driven bone formation (Zuk, unpublished observations). In addition, it remains unknown the effect of such a powerful growth factor as BMP2 would have on the craniofacial arena in very young children. The question becomes how do we augment the ability of an adult stem cell, like the ASC, to make large quantities of bone within a craniofacial defect like a cleft palate without such growth factors? Promising results may be linked to three distinct approaches or a combination of them.

The "Scaffold-Driven" Approach—Biomimetic Apatites

Efficient use of 3D scaffold systems in bone repair is dependent upon their bond-bonding or bioactive ability. Although scaffolds such as PLGA or PLA composites provide the reconstructive surgeon with a biodegradable platform for stem cell adhesion and differentiation, their bioactivity can be limited. However, studies have suggested that their bioactivity can be strengthened through the formation of a layer of HA at the bone-implant interface (122,123). Several HA materials for use in bone differentiation have been developed within the last 20 years and are thought to possess superior *in vivo* bioactivity. However, much excitement has been generated regarding the osteoinductive capacities of biomimetic apatite coatings. Typically created through the immersion of 3D scaffolds in ionic solutions with compositions similar to blood plasma—called Simulated Body Fluids—biomimetic apatites are composed of plate-like crystals of calcium phosphate capable of coating the entire 3D scaffold architecture (124,125). An improvement on biomimetic apatites has recently been presented by Wu and colleagues through their development of accelerated biomimetic approaches that dramatically shorten the time required for coating from approximately 2 weeks to 2 days (126,127). Such convenience may make the accelerated biomimetic apatite more attractive for *in vivo* applications such as bone healing. In support of this, accelerated apatite coatings have been shown by Wu and his group to promote bone in-growth and differentiation of preosteoblasts and bone marrow stem cells and to enhance direct bone to bone contact (125). Recently, accelerated apatites have also been shown to promote the osteogenic capacity of ASCs. In a landmark paper by Cowan *et al.,* murine ASCs seeded onto accelerated apatite coated PLGA scaffolds were found to heal critical-sized cranial defects without the need for exogenous stimulation such as BMP2 treatment (128). Although the ASCs used were murine and no further studies using human ASCs have been presented, these results remain exciting because they show the reconstructive surgeon that methods other than conventional growth factor stimulation may be used to induce stem cells to make and heal bone.

The "Cell-Driven" Approach—The Pediatric Stem Cell

Despite all that we know of the adult stem cell, we still know very little about how their age affects their differentiative capacity. The use of a pediatric stem cells in the repair of craniofacial defects should be intuitive since repair of the defect would require the child's own stem cell. With regards to the ASC, it would be relatively simple for the craniofacial surgeon to extract a small amount of adipose tissue from the child using a simple syringe. This could easily be done during one of the many preparatory procedures that often precedes major craniofacial reconstruction. These pediatric ASCs (pedASCs, *i.e.*, under 5 y) could be expanded in the lab and combined with the best possible scaffold for implantation into the defect. Yet, there is no current information available that studies pedASCs at an in-depth level.

The "Gene-Driven" Approach—Molecular Signaling Within the Stem Cell

Although the adult stem cell researcher has learned much of the ultimate downstream genes involved in bone differentiation (*i.e.*, Runx2/Cbfa1, Osterix), we are only beginning to understand the upstream mechanisms that control them. Numerous studies have elucidated possible signaling pathways downstream of BMP2 induction and how these pathways may affect osteogenic gene transcription (129–131). However, it is possible that osteogenesis in ASCs is under an alternate

signaling pathway. In fact, very little is known about signal transduction pathways in adult stem cells like ASCs. Jaiswal *et al*. (132) have examined the role of MEK-ERK signaling in deciding adipogenic and osteogenic fates in MSCs. ERK pathways and there role in obesity and adipogenesis have been examined in ASCs along with the role of MAPK signaling in ASC proliferation, migration, and apoptosis (133–135). However, the number of these studies does appear to be increasing as researchers turn to gene therapy approaches in the hopes of understanding and alleviating the disease state. For example, significant bone regeneration in a rabbit calvarial model has been measured upon implantation of MSCs transduced with Sonic Hedgehog (Shh)—a key protein involved in craniofacial morphogenesis (136). Although these results are promising, the stem cell population must be carefully considered as Shh-expressing ASCs were capable of regenerating bone within a calvarial defect but also appeared to induce the formation of large cyst-like structures. Canonical and noncanonical Wnt signaling pathways have also come under focus because of their well-known role as regulators of embryologic patterning, stem cell fate and mesenchymal differentiation (137). Observations linking the LRP5 gene mutation and osteoporosis-pseudoglioma syndrome have suggested a connection between Wnt signaling and bone formation (138). Consistent with this, work in MSCs has linked Wnt3a induced signaling to a suppression of bone formation *in vitro* and *in vivo* (139). In contrast, increased bone regeneration in both mandibular and calvarial defects has been observed in MSCs isolated from craniofacial tissues overexpressing Wnt4 (140). Analysis of these Wnt4-transduced MSCs identified a specific increase in p38MAPK phosphorylation suggesting that increased activity of this MAPK kinase may act to promote MSC-driven bone formation within the defect. Similar to this, microarray analysis of developing orofacial tissues has identified the differential expression of a number of MAPK pathway genes, suggesting that this pathway may play a role in craniofacial development (141). Together, these studies suggest that stem cell-directed bone within craniofacial defects might be augmented not through upstream growth factors but through the careful and directed manipulation of their downstream signaling paths. Finally, in stem cells like MSCs, adhesion significantly affects osteogenic differentiation with differential effects being attributed to the actual substrate (142). This effect is likely to be controlled at many levels including interactions between the substrate and integrin complexes (143,144). Because numerous signaling pathways, including the MAPK cascade, can be induced through integrin—matrix interactions in a variety of cells (145), it is not unreasonable to hypothesize the design of scaffolds that mimic the effect of growth factors through adhesion-based mechanisms, mediating signaling through specific "pro-osteogenic" signal transduction pathways.

"State of the Field"—A "Call to Arms" to the Adult Stem Cell Researcher

It seems like everyday we read about huge advances in the field of ES research detailing daring approaches in the application of these stem cells to the clinical world. The question is where is the adult stem cell researcher? True advances in the medical and scientific world using adult stem cells like the ASC will not be achieved by playing it "safe." In the modern marketing world, we hear phrases like "kick it up a notch" and "think outside the box." The adult stem cell researcher needs to take these phrases to heart and look outside conventional fields for inspiration and knowledge. The combination of the stem cell researcher with the biomaterials engineer has been an example on how what appears to be an unconventional collaboration can advance the scientific community and benefit the general public. Who knows where these relationships will take us? But one thing is for sure—collaboration brings people together who look at the world in different ways. From these unions, we can propel the fields of adult stem cell biology and craniofacial tissue engineering into a whole new realm—where all things are possible.

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