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The Role of Skeletal Stem Cells in the Reconstruction of Bone Defects

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Abstract

Craniofacial surgery, since its inauguration, has been the culmination of collaborative efforts to solve complex congenital, dysplastic, oncological, and traumatic cranial bone defects. Now, 50 years on from the first craniofacial meeting, the collaborative efforts between surgeons, scientists, and bioengineers are further advancing craniofacial surgery with new discoveries in tissue regeneration. Recent advances in regenerative medicine and stem cell biology have transformed the authors' understanding of bone healing, the role of stem cells governing bone healing, and the effects of the niche environment and extracellular matrix on stem cell fate. This review aims at summarizing the advances within each of these fields.

Keywords

Bone; craniofacial; reconstruction; stem cell

In 1967, Paul Tessier presented his work in Hospital Foch among plastic surgeons, neurosurgeons, pediatric surgeons, and ophthalmologists, and the speciality of Craniofacial Surgery was born. Tessier's work with craniosynostosis and his description of the "orbit utile of Tessier" revolutionized how congenital facial and cranial abnormalities were managed.[1] Since then, the field of Craniofacial Surgery has continuously moved at the forefront of biological and technological advances. Innovation ranging from 3-dimensional (3D) reconstruction of computed tomography scans, to the advent of 3D printing, now allows patient-specific application of principles set out by Tessier, Gilles, Le Fort, and Virchow to provide the optimal tailored aesthetic and functional outcomes.[2,3] These techniques, coupled with new discoveries in identifying stem cells and factors capable of

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osteogenic differentiation and integration of biological scaffolds synergistically, provide surgeons with the potential to regenerate bone in vivo.[4] However, these new discoveries have also highlighted the importance of the stem cell niche and how, through disease, these niche systems can fail to support stem cells.[5]

While the technology used by craniofacial surgeons has expanded exponentially over the century, the common pathologies leading to critical sized bony defects remain unchanged. A continued challenge to any craniofacial surgeon is reconstructing these defects. A critical sized bony defect is the one that lacks enough bony tissue to heal spontaneously.[6] The actual size of the defect depends on factors that affect healing such as: the location of the defect and the health of the surrounding bone and soft tissue from infection, diabetes, radiotherapy, and osteoporosis.[7,8] This review will provide an overview of the current clinical landscape as well as recent developments in stem cell medicine that could dramatically enhance the surgical tool set for not only reconstructing but regenerating craniofacial defects.

STRUCTURE OF THE CRANIOFACIAL SKELETON

Craniofacial bones are different from long bones in their developmental origins and osteogenic programs and structures. Craniofacial bones are flat and develop mainly through intramembranous and endochondral ossification. Most craniofacial bones are derived from the cranial neural crest, whereas long bones are derived from trunk mesoderm.[9–11] At the macroscopic level, outer cortical bone surrounds porous inner trabecular bone. Microscopically, cortical bone is composed of repeating osteon units containing collagen fibers and calcium-phosphate crystals, whereas cancellous bone is an interconnecting framework of trabeculae with a surrounding marrow space. A single osteon unit consists of concentric layers of collagen fibers, called lamellae, running perpendicular to a central canal containing blood vessels and nerves.[12] Craniofacial bones have little marrow and are sheathed by periosteum and dura.

CRITICAL SIZE DEFECTS IN THE CRANIOFACIAL BONE

Critical sized bony defects can occur in pediatric, dysplastic, oncological, and traumatic settings. Within the pediatric setting, large sized calvarial defects are particularly difficult to treat as the developing cranium’s anatomy changes with age and maturation.[13] Bone characteristics change with age, with 50% of pediatric autologous cranioplasties undergoing resorption compared with 6.5% in adult populations.[14,15] When resorption occurs there is a need for either further reconstruction often with additional autoplastic bone grafts or the use of alloplastic substitutions.

In the oncological setting, radiation of tissues leads to reduced ability of the underlying bone to regenerate. Indeed, radiation can lead to osteoradionecrosis. Radiation negatively affects bone remodeling as it reduces osteoblast and skeletal stem cell numbers and cytokine signalling in vitro.[16] When using biomaterials, the longer the delay between the irradiation and the implantation of the biomaterial, the lower the rate of failure.[17]

Any trauma to the head requiring cranioplasties is at increased risk of failure if the defect is large with poor soft tissue coverage and infection.[18] When alloplastic implants are used,

their risk of failure is increased when there is a connection with the orbit or extension into the sinuses, both introducing infection.[19] The limitations of alloplastic materials underline the need for further research in developing autologous means to reconstruct critical cranial defects.

CURRENT LIMITATIONS WITHIN RECONSTRUCTION

There are 2 broad strategies when reconstructing craniofacial defects. These are autologous sources of tissue and alloplastic sources of bone reconstruction. To summarize, autologous sources can be subdivided into vascularized and nonvascularized. Autologous vascularized strategies include free flaps such as free vascularized fibular grafts for the reconstruction of the mandible after oncological resection. Autologous nonvascularized strategies include calvarial outer table bone grafts used in cranial reconstruction after trauma. While both vascularized and nonvascularized bone grafts can be used, they exhibit different properties. Importantly, vascularized bone grafts have a much lower risk of infection.[20]

The limitations with using both sources of autologous tissue for bony reconstruction are donor site morbidity and lack of tissue available for the reconstruction.[21] As mentioned before, the organic nature of the autologous bone means its properties are dynamic. Over time, the grafted bone, in an attempt to remodel, may resorb, reducing its size and altering its mechanical strength.[22,23]

The second strategy for craniofacial reconstruction is allogenic sources, which can be divided into synthetic and organic categories. Within allogenic synthetic sources there is a wide choice of materials from titanium to hydroxyapatite. Allogenic organic sources include allograft bone from bone banks as well as xenograft bone and possess some advantages over synthetic sources. Although synthetic sources, such as titanium, may possess superior mechanical properties, they are limited in their ability to adapt, integrate, and develop with the surrounding bone.

Ideally, both autologous and synthetic materials could be applied synergistically to optimize anatomically appropriate bone regeneration. This underlies the fundamental principle of plastic surgery to restore “form and function.” For example, the combination of autologous stem cells applied to a synthetic scaffold which mimics the ideal osteogenic extracellular matrix (ECM) may allow for the regeneration of site-specific, functionally correct, bone.

BONE HEALING IN THE CRANIOFACIAL SKELETON

The process of skeletal repair is controlled by stem and progenitor cells that are phenotypically distinct.[24–27] The histology of the ECM depends on the embryonic germ layer of origin of that bone.[28–30] Although there are differences in both intramembranous and endochondral ossification, there are some similarities. Following trauma, injury sites in intramembranous and endochondral bones are vascularized to a similar extent; become populated by resident skeletal lineage-derived cells; show evidence of a bony matrix that undergoes extensive remodeling; and heal the defect within a similar time frame.[31] The periosteum has been proposed to play major roles in the bone healing process.

After an injury, an acute inflammatory reaction can be seen within the periosteum. Subsequently, periosteal progenitor cells begin to proliferate, which leads to a thickening of the periosteum. The periosteal cells then differentiate to form osteocytes or chondrocytes to repair the injury site. In other studies, the dura mater has been reported as a source of healing calvarial bone following injury.[32] Critical sized defects in the craniofacial bones caused by pathological conditions or trauma will not heal spontaneously if no intervention is made. Contributing factors may include injury size, age, systemic conditions, nutrition, and infection. Over time, the unhealed injury site is filled with fibrous tissue instead of bone.

THE SKELETAL STEM CELL ROLE IN CRANIAL DEVELOPMENT

Skeletal formation in the craniofacial region and long bones is derived from different embryonic germ layers and forms bone via different processes, as mentioned before.[33,34] Due to their different embryonic origins, long bones and craniofacial bones are likely to have their own unique stem cells. In mice, the long bone skeletal stem cell has been recently identified, but remains elusive in humans.[24] In mice, studies have shown a single skeletal stem cell can give rise to bone, cartilage, and stroma of the long bones. During injury, these mouse skeletal stem cells (mSSCs) are activated. In the fracture callus of long bones, a highly potent regenerative progenitor cell type is activated and is responsible for skeletal fracture healing [26].

In the craniofacial complex, studies in mice have shown that cells expressing Axin2 and Gli1 in the suture mesenchyme form the bones that make up the craniofacial structure.[35,36] In calvarial injury models, these Axin2 and Gli1 expressing stem cells undergo osteoblast differentiation for skeletal repair.[35,36] In mice, additional studies have shown that pluripotent stem cells can be used to regenerate bone in calvarial critical sized defect.[37–39] In humans, the only solution for such craniofacial injuries is to undergo reconstructive surgery.[40]

THE SKELETAL STEM CELL NICHE

Stem cells reside in complex microenvironments called niches. The stem cell niche maintains stem cells in a stem-like state and can also direct the stem cell's ability to self-renew, expand, and differentiate. Aberrancies in the microenvironment can lead to abnormal cell growth and differentiation manifesting in cancer.[41] In mice, the SSC forms bone, cartilage, and stroma. Under defined conditions, the mouse SSC niche can be manipulated to direct the SSC to a cartilage fate. In vivo studies showed that treatment with bone morphogenic protein-2 and inhibition of vascular endothelial growth factor using soluble vascular endothelial growth factor receptor directs the mSSC toward a cartilage fate, perhaps providing a therapeutic avenue for cartilage repair.[24] Contrarily, defects in the skeletal stem cell niche can lead to aberrant gene expression and ultimately manifest in skeletal deformities and anomalies. In diabetic mouse models, high serum levels of tumor necrosis factor-alpha repressed the expression of Indian hedgehog, impairing mSSC expansion and fracture healing. This deficiency was shown to be reversible by directly delivering Ihh to the niche or fracture site.[5]

In the craniofacial complex the calvarial sutures serve as growth centers and play a crucial role in the healthy development of the craniofacial skeleton. The suture mesenchyme is believed to act as a niche for resident skeletal stem cell.[35] For the sutures to function as growth centers they must remain patent in an unossified state. Craniosynostosis is a condition wherein the calvarial sutures fuse prematurely. Craniosynostosis is well characterized and is associated with facial deformities, cognitive problems, and problems with vision and hearing.[42] The interaction of fibroblast growth factor receptor 1 with Wnt is known to govern craniofacial skeletal stem cell fate.[43] Studies in mice have shown that a suture niche treated with Wnt antagonists sufficiently downregulates Wnt signaling and causes sutures to fuse prematurely. Conversely, when supplied with Wnt the sutures remained patent, suggesting the suture niche is tightly regulated by Wnt signaling.[44] Mice with haploinsuffcient Twist1, a Wnt target gene, exhibited craniosynostosis and a reduced number of Gli1+ stem cells.[36]

CURRENT STEM CELL-BASED THERAPIES

Several stem cell-based therapies have emerged for the treatment of critically sized calvarial bone defects. Interestingly, these are not solely bone derived, and may be used for a variety of clinical applications. Beginning in 2002, with a seminal paper from Zuk et al, who identified adipose-derived stem cells (ADSC) from human adipose tissue, both human and mouse ADSC have been used in healing bone defects within the calvarium.[38,45,46] Adipose-derived stem cells are isolated easily from the stromal fraction of lipoaspirate and can be induced into bone tissue.[47]

Clinically, these cells have been used for dental, mandibular, and other craniofacial bone reconstructions, often combining scaffolds derived from synthetic polymers or bone grafts with cell signaling molecules like BMP-2.[48–53] Additionally, ADSCs can differentiate into bone, fat, and myogenic cell lines under appropriate inductive conditions, making them an attractive candidate for areas of problematic soft tissue loss as well.[54,55] However, though many exciting animal models exist, testing in human patients is limited.[48,49,51,52,54–58] Excitingly, the studies that do exist report the remarkable ability of ADSCs to heal critically sized calvarial bone defects, and demonstrate their ability to recruit neovasculature to newly formed bone.[59,60] Additionally, ADSCs can form mature bone, and with the assistance of scaffolds, that can receive dental implantations for major reconstructive surgery.[61]

Limitations to this technology currently include the need to expand cells in culture with potentially mutagenic cell signaling molecules. However, the expansion of “GMP” clean room technologies to allow safe and sterile prolonged cell culture of human stem cells may eventually allow for FDA approval and safe clinical trials.[61,62] Regardless, ADSCs represent an exciting resource for cell-based therapy.

An alternative to ADSC are muscle derived stem cells (MDSC). While MDSC pose greater donor site morbidity, they have been shown to have potential uses in healing of calvarial defects in vivo in rats.[63] Human MDSC, when transfected with lentivirus BMP2, showed osteogenic potential in vivo in healing critical sized defects in nude mice. This suggests that

human MDSC are a form of mesenchymal stem cells with the ability to undergo osteogenic differentiation in the presence of BMP2.[64]

A third type of adult stem-cell therapy for bones are bone marrow-derived mesenchymal stem cells (BMSCs). Bone marrow-derived mesenchymal stem cells were widely used in bone regeneration research long before the discovery of the multipotent abilities of ADSCs or MDSC.[65] In craniofacial tissue engineering, BMSCs have similar capabilities to ADSCs, and improve healing in critically sized calvarial defects.[66] However, their clinical applicability is much reduced due to the superiority in safety and donor site morbidity in ADSC harvest compared with BMSC harvest. Nevertheless, BMSC research continues as these cells still represent a source of autologous stem cell therapy.[67]

To date, BMSCs have been proven to augment fascial healing, which may lend itself to meningeal tissue research and speed time to skin wound healing. Bone marrow-derived mesenchymal stem cells are capable of regenerating bone and tooth anatomy.[66,68] Most recently, research focuses on the use of BMSCs in local tissue harvest, donation, and reconstruction. Notably, third molar BMSC harvest is a promising technique that lends itself to dental reconstructive surgery.[69] Also, location and age of BMSCs appear to be increasingly important. Fetal BMSCs have greater proliferative capability, and in select patients (such as the aged and diabetics) with defective autologous stem cells, these may be an alternative resource. [70,71]

Even prior to the use of BMSCs, human embryonic stem cells (hESCs) were known to be capable of de novo bone formation.[72] These cells, derived from the inner cell mass of a human blastocyst, are totipotent and capable of differentiating easily into any of the major embryonic germ layers.[72] For the purposes of bone tissue engineering, hESCs must first be differentiated into BMSCs or similar mesenchymal cells. From there, the cells are induced to differentiate into osteoblast-like cells.[73] While hESCs have great potential in tissue engineering research, they continue to pose a problem for human patients due to their tumorigenicity and the risk of contamination by other cell types in culture. Because hESC-derived cells represent an allograft, they are also subject to eventual engraftment destruction. [73–75] Other embryonic and fetal cells have been isolated for the purposes of bone tissue engineering; however, they have the same limitations as hESC-derived cells.[70,71,76]

Due to the political, religious, and ethical controversies surrounding hESCs, induced pluripotent stem cells (iPSCs) are now more commonly studied in bone tissue allograft generation. Like hESCs, iPSCs can generate any of the three embryonic germ layers, and can also, after mesenchymal differentiation, become bone. Recently, biomaterial papers have focused on generating both osteoblast and osteoclast populations from iPSCs to promote bone regeneration.[77] This may improve the quality of newly formed bone. Lastly, local stem cell populations, such as those in the hair follicle, are capable of regenerating hair lines and potentially other important facial hair structures like eyebrows; which again can be harnessed in craniofacial reconstruction.[78] In facial feminization surgery, this has been a useful technique, which may be translatable to burn patients, and certain postsurgical patients with large soft tissue defects.

In terms of the quality of bone generated from these varieties of auto and allografts, it appears strong mature bone may be formed, though without many of the native properties of local tissue.[70,71,76] For instance, osteoblasts and osteoclasts will not exist in normal ratios.[74] Interestingly, cell signaling molecules that induced osteoclast behavior, or the use of impregnated scaffolds, tend to create strongermore native appearing bone.[74,77] Also, diploic veins are not regenerated in healed calvarial defects. Nonetheless, regenerated mandibles can receive dental implants, and the amount of area healed is significantly improved in treated tissue compared with control.[59,76,79]

HOW SCAFFOLD STRUCTURE AFFECTS STEM CELL FATE

It has been reported that scaffold structure could play a more critical role in stem cell fate than scaffold composition.[80,81] Scaffolds with nanofibrous morphology can drive stem cells toward osteogenic lineage in the absence of osteogenic supplements. Nanofibers provide fibrous adhesion sites for stem cells and affect the shape of and communications among cells which drives them to an osteoinductive lineage.[82]

Three-dimensional structure is a pivotal component, and the challenge is to replicate native bone as closely as possible, as each pore and grove influences communication signals and thus stem cell fate.[83] Many technologies for designing scaffolds attempt to duplicate the ideal fibre size, pore structure, grove topography, and other important 3D features identical to that of native ECM. This is critical for cell infiltration, migration, and communication.[84]

Stem cell morphology and cell surface characteristics are involved in lineage specification and function, and therefore scaffolds that can direct cell shape can also affect their function.[85] Physiologically, it is similar to native stem cell niches directing conformational changes to influence cell purpose. The nanotopography is not only important for influencing stem cell shape, but also the specific surface grooves and ridges of scaffolds serve as binding sites in integrin signaling interactions. In this way, the scaffold structure directs physical and biochemical cues.[86,87]

Stem cells could respond to shape-dependent biophysical cues via cell signaling, receptor and ligand interactions, and protein–protein interactions. Various mechano-transduction pathways have been proposed, such as the MAPK, which, when coupled with growth factor-mediated signaling pathways, regulate stem cell fate.[84] While there have been important strides in understanding these communications, more research is needed to further understand the cell signalling interactions. In addition, improvement of technologies for scaffold designs is needed to be able to more closely recreate native structures and thus better influence interactions and stem cell fate.[88]

THE CURRENT LIMITATIONS TO SKELETAL STEM CELL APPLICATION IN CRANIOFACIAL SURGERY

One major consideration in any stem cell-mediated therapeutic approach is the health of the stem cell niche. As described earlier, the SSC niche can be affected by diseases such as diabetes.[5] Aberrant signaling via suppression of Ihh in diabetes is just one of a multitude of

examples where disease leads to loss of critical signaling required for stem cell proliferation. Within the scope of craniofacial surgery, we have mentioned age, systemic factors, radiation, and infection as being key factors in healing critical sized bone defects, but how do these factors affect skeletal stem cell fates via the niche environment? While the identification of the stem cells within each tissue type has added hugely to our understanding of the complex interplay between the stem cells and their surroundings the role of the niche is not fully understood. In order to maximize the benefits of stem cells, we need to first understand the niche. Once this is accomplished we may be able to deconstruct it, reconstruct it, and finally use the niche to reprogram diseased tissues.[89] The most cutting-edge methods now employed to understand the niche are based on single-cell analysis (deconstruction) and organoid study (reconstruction).[90–92]

CONCLUSION AND FUTURE PERSPECTIVES

Critical size cranial defects remain a challenge for reconstructive surgeons. Advancements in our understanding of how calvarial bones heal, the identification and isolation of the SSC, our knowledge of niche factors, and the design of scaffolds that mimic the native ECM have all brought us closer to the possibility of regenerating de novo cranial tissue. With close collaboration and crosstalk between bioengineering, materials science, stem cell biology, and craniofacial surgery, our ability to regenerate whole cranial bones to heal critical sized defects in patients is close to realization.

From the work of Vacanti to the work of Warnke we have seen advancements in the use of tissue engineering to design and develop de novo, in vivo heterotopic tissues using scaffolds.[93,94] The potential for 3D printing of patient-specific, osteogenic ECM scaffold infused with the patient's own ADSC or BMSC could allow for complete reconstruction and neo-vascularization of the bone.[83,95–98] We hypothesize that soon; we will be able to deliver and control growth factors within scaffolds, which at specific times allow for differentiation of stem cells into the tissue required to reconstruct any craniofacial bony defect. Within this review we have focused on the use of skeletal stem cells in the reconstruction of bone defects.

However, bone healing is only possible with adequate soft tissue coverage. Facial transplantation remains limited with poor outcomes and a lifetime of immunosuppression.[99] The potential to reconstruct not only bone but neurovascular and soft tissue compartments would allow for complete orthotopic autologous craniofacial regeneration. To regenerate tissues from all germ cell layers, we would first have to identify the resident tissue-specific stem cells and their native niches.[100] This would ultimately replace the need for composite tissue transplantation in craniofacial reconstruction.

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