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REVIEW ARTICLE

Stem cells, growth factors and scaffolds in craniofacial regenerative medicine

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Abstract Current reconstructive approaches to large craniofacial skeletal defects are often complicated and challenging. Critical-sized defects are unable to heal via natural regenerative processes and require surgical intervention, traditionally involving autologous bone (mainly in the form of nonvascularized grafts) or alloplasts. Autologous bone grafts remain the gold standard of care in spite of the associated risk of donor site morbidity. Tissue engineering approaches represent a promising alternative that would serve to facilitate bone regeneration even in large craniofacial skeletal defects. This strategy has been tested in a myriad of iterations by utilizing a variety of osteoconductive scaffold materials, osteoblastic stem cells, as well as osteoinductive growth factors and small molecules. One of the major challenges facing tissue engineers is creating a scaffold fulfilling the properties necessary for controlled bone regeneration. These properties include osteoconduction, osteoinduction, biocompatibility, biodegradability, vascularization, and progenitor cell retention. This review will provide an overview of how optimization of the aforementioned scaffold parameters facilitates bone regenerative capabilities as well as a discussion of common osteoconductive scaffold materials.

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Introduction

Large craniofacial skeletal defects secondary to trauma, congenital condition, or cancer resection pose serious challenges to reconstructive surgeons. Extensive defects which prevent spontaneous re-ossification are termed 'critical-sized' and often require complex reconstructive approaches (Fig. 1A).[1] Repair of these defects has traditionally required autologous bone grafts from a variety of sources, including cranium, tibia, rib, and iliac crest (Fig. 1B).[2,3] These procedures, although they have seen success clinically and are currently the gold standard of care, necessitate a second surgical site with a significant risk of morbidity. In particular, undesirable sequelae at the donor site include infection, bleeding, pain, swelling, unanticipated fractures, and injury to adjacent critical structures.[4–6] Additionally, autologous bone graft procedures have been complicated by unpredictable graft resorption rates, limited supply of autologous bone, and rapid bone remodeling in young children.[2,3,7]

Alternatives in the alloplast category, including demineralized bone matrix, bone ceramics, porous polyethylene implants, and various other polymers, have seen variable success. However, they generally carry a greater risk of infection than autologous bone grafts and are more likely to fail over time.[8–12] Permanent methods of rigid fixation utilizing metals or metal alloys suffer similar limitations in addition to integrating poorly with the surrounding tissue.[13] Because craniofacial reconstructive surgeries are often performed on children (Fig. 1) who require repair capable of accommodating natural growth and development, permanent rigid fixation is not the most favorable alternative.

Biocompatible implants that augment natural bone-regenerative capabilities currently represent the most promising and versatile approach to repairing critical-sized craniofacial defects.[14] This tissue engineering-based strategy generally involves three key elements: osteoconductive scaffolding, stem cells, and growth factors (Fig. 2). These three elements allow osteoblastic and endothelial progenitor cell differentiation, bone formation, and integration with surrounding bone tissue even in large defects.[15] Osteoblastic stem cells within an osteoconductive scaffold provide the possibility of a tailored three-dimensional space for bone growth. Osteoblastic differentiation can be induced by a variety of osteoinductive growth factors both *in vivo* and *in vitro*.[16] Finally, efficacious bone regeneration requires integration with surrounding tissue, including vascularization, fusion of the implant with autologous bone without fibrous tissue at the bone-implant interface, and eventual complete replacement of the scaffold with new bone.[17–19]

The goal of achieving these prerequisites has challenged tissue engineers to choose the optimum combination of cell types, scaffold properties, and growth factors. The process is inherently complex and multidisciplinary due to requisite collaboration between molecular biology, materials science, surgery, and mechanical engineering.[20] This review will explore current progress toward achieving reliable repair of craniofacial defects using osteoconductive scaffold and osteogenic stem cell-based tissue engineering.

Stem cells used for bone regeneration

Irrespective of craniofacial bone defect size or complexity, healing is fundamentally dependent on the presence of osteogenic and vasculogenic precursor cells in surrounding tissues.[21] These precursors migrate to the injury site and differentiate into osteoblasts and endothelial cells, promoting bone formation and vascularization.[22] In recent years, clinical reports have suggested that stem cell supplementation may work synergistically with this natural progenitor cell migration and differentiation to produce the best results in healing critical-sized bone defects.[22–31]

Several stem cell types have been used both *in vitro* and *in vivo* to produce new bone (Fig. 3). Bone marrow-derived mesenchymal stromal cells (BMSCs) are increasingly being applied to craniofacial defect repair, and several studies have substantiated their effectiveness as osteoblastic precursors in critical-sized defect reconstruction.[32–34] A recent phase I/II clinical trial determined that $CD90^{+}$ osteoblastic BMSCs and neovascularization-inducing $CD14^{+}$ monocytes and macrophages seeded onto a β-tricalcium phosphate (β-TCP) scaffold provided a viable treatment for patients with severe maxillary bone deficiency.[35,36] When compared with scaffold alone, the progenitor cell-seeded scaffold treatment showed a higher proportion of regenerated viable, highly vascularized, and mineralized bone in addition to a lower proportion of residual β-TCP particles four months postoperatively.[35] Mesenchymal stem cells derived from umbilical cord blood have also been used successfully, in conjunction with poly-lactic co-glycolic acid (PLGA) implants, to heal critical-sized alveolar cleft defects in a swine model. Investigators reported no inflammation and better bone quality than autologous bone graft from the iliac crest by CT volumetric and histological analysis.[37] However, despite its success, the use of BMSCs is limited by finite supply and the morbidity associated with procurement procedures.[38]

Adipose-derived stem cells (ADSCs) represent a promising alternative to BMSCs in that they are more plentiful, less painful to harvest, and easily expandable.[39] ADSCs have showed similar osteogenicity to BMSCs, with certain subpopulations demonstrating enhanced tendency toward osteoblast differentiation and others successfully induced through gene therapy.[34,40] The necessity for invasive procedures during harvesting still constrains ease of access to ADSCs and the scope of their clinical significance.

Urine-derived stem cells (USCs), which can be obtained from voided urine and require no invasive procedures, have recently garnered a great deal of attention in the bone tissue engineering community as a promising, but still poorly studied, alternative stem cell source. Research regarding USCs is still in its infancy, but recent studies by Guan et al have demonstrated their applicability to bone regeneration.[38,41–43] USCs are biologically similar to ADSCs and are capable of osteogenic differentiation *in vitro*.[43] Furthermore, USCs have successfully differentiated into osteoblasts via calcium silicate ion induction of the Wnt/β-catenin signaling pathway.[38] They have also been shown to be compatible with both calcium sulfate/PLGA composite and β-TCP scaffolds.[38,42]