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Craniofacial Bone Tissue Engineering

Derrick C. Wan, MD, Randall P. Nacamuli, MD,
Michael T. Longaker, MD, MBA*

*Stanford University School of Medicine, 257 Campus Drive West,
Stanford, CA 94305-5148, USA*

Since the turn of the millennium, the annual growth in United States health care expenditure has increasingly outpaced the annual growth in gross domestic product by ever-increasing margins [1]. Current expenditures exceed $1.5 trillion, with unabated demand, burgeoning costs, and an aging population contributing to this predicament. Data from the United States Healthcare Utilization Project revealed that over 1 million skeletal-related procedures were performed in 2002, with 16,338 craniotomies/craniectomies and 32,043 post-traumatic facial reconstructions accounting for over $585 million in medical care [2]. Extending these procedures to include the correction of congenital craniofacial anomalies and malformations only serves to further underscore the biomedical burden associated with the treatment of skeletal defects.

Large bone defects resulting from trauma, tumor resection, nonunion of fractures, and congenital malformations are common clinical problems in craniofacial surgery, which have proven difficult to remedy. Current surgical techniques have used, in various combinations, autogenous, allogeneic, and prosthetic materials to achieve bone reconstruction [3]. However, the multitude of dissimilar solutions currently in practice highlights the fact that an ideal solution has yet to be defined. Autogenous bone grafting generally has yielded favorable results, but this practice is limited by donor-site morbidity and the amount of bone that may be harvested [4,5]. In situations where insufficient autogenous bone exists, use of allogeneic bone may also be used. This approach, however, is also beset with a multitude of concerns, chief among which include infection, immunologic rejection,

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* Corresponding author.
E-mail address: Longaker@stanford.edu (M.T. Longaker).

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and graft-versus-host disease [5]. Alternative materials have therefore been developed to assist in bone reconstruction, with metal alloys, glass, plaster of paris, polymethylmethacrylate, and, more recently, biodegradable scaffolds all being investigated [3,6,7]. Discouragingly, none of these modalities have yet to prove a consummate tool for craniofacial bone reconstruction.

By combining decades of clinical experience with recent studies in molecular, cell, and developmental biology, however, the field of bone tissue engineering has rapidly become a practical approach to the treatment of many craniofacial skeletal defects. Mechanical-based (distraction osteogenesis) and cell-based (multipotent mesenchymal cell) modalities have garnered particular attention not only from an investigational stand point but also from their present-day clinical applications [8,9]. Since its general introduction over 50 years ago, distraction osteogenesis has revolutionized the treatment of many congenital hypoplasias afflicting children [10,11]. As a form of endogenous tissue engineering, distraction osteogenesis has spread rapidly throughout the field of craniofacial reconstruction and is currently the treatment of choice for several midface and mandibular deformities [12–14]. Like distraction osteogenesis, regenerative medicine also has the potential to transform the field of craniofacial skeletal repair through a cell-based approach to engineer bone. At its core, regenerative medicine incorporates the use of multipotent building blocks combined with molecular and environmental cues for the repair of damaged or diseased tissue. Recent investigations have focused upon the post natal mesenchymal stromal cell population that has been demonstrated to possess the ability to differentiate down multiple lineages in appropriate environments [15]. As studies continue to define the true nature of these cells, their potential for clinical application already has been demonstrated in the report of a calvarial defect reconstruction from Germany in 2004 [9].

Considering the large biomedical burden skeletal reconstruction comprises, distraction osteogenesis and cell-based tissue engineering increasingly will become critical modalities for craniofacial bone reconstruction. Both modalities carry the potential for generation of novel bone without the attendant limitations of current allogeneic and prosthetic strategies. This article focuses on these two significant paradigms for craniofacial bone tissue engineering and present emerging knowledge from recent investigations to elucidate the biologic underpinnings of these processes.

Distraction osteogenesis

Reconstruction of skeletal hypoplasias involving the mandible, maxilla, midface, orbits, and cranial vault continues to present a significant challenge to contemporary craniofacial surgeons. Children who present with bony insufficiencies often suffer from a host of disabilities ranging from severe airway compromise to malocclusion and a dysfunctional bite. Traditional approaches at reconstruction using osteotomies and bone grafting can be

associated with unsatisfactory outcomes and significant short- and long-term morbidities. Since the adoption of distraction osteogenesis to the correction of craniofacial skeletal hypoplasias, however, more favorable results have been obtained and this modality rapidly has become the treatment of choice for several midface and mandibular deficiencies.

Distraction osteogenesis is a powerful form of endogenous tissue engineering, promoting bone formation through the gradual separation of osteogenic fronts. Despite its recent application to craniofacial surgery, the fundamental principles of distraction osteogenesis have existed since the early twentieth century [16]. In 1956, Ilizarov [11,17,18] demonstrated this modality could be consistently applied to long bone reconstruction with acceptable morbidity. The first translation to intramembranous bone of the craniofacial skeleton was established in 1972 using a canine model and, in 1989, McCarthy [8] performed the first human mandibular distraction [19]. Since that landmark description, this technique has now become a standard tool for craniofacial surgeons to achieve clinically significant midface and mandibular advancement.

As elaborated by Ilizarov, distraction osteogenesis incorporates rigid fixation with a several day latency period, followed by gradual distraction and stable fixation until radiographic and clinical assessment demonstrates the formation of a robust, mineralized regenerate [11,17,18]. Despite ever increasing experience, however, significant complications nonetheless continue to plague surgeons performing this procedure; overall morbidity rates as high as 35% have been described [20]. Most commonly, soft-tissue infection, osteomyelitis, and pin-tract infection or loosening secondary to daily manipulation of exposed devices have been reported. Patient discomfort and complications related to compliance also contribute to overall morbidity. Lastly, fibrous nonunion, permanent inferior alveolar nerve injury, and relapse of the original condition typically within the first 6 months following distraction remain significant considerations in the postoperative period [20]. In the face of such concerns, however, overall results remain acceptable, with surgeons reporting good or excellent results in over 86% of patients [21,22].

With a goal to further optimize these clinical outcomes and minimize associated complications, recent investigations have endeavored to better characterize the mechanisms guiding successful bone formation in the regenerate. These studies have focused primarily on the mechanobiology and molecular biology of successful osteogenesis during guided distraction. Large animal models, including canine, ovine, and lupine species, have been used traditionally in these investigations to delineate the histologic and ultrastructural changes associated with robust bone deposition [19,23–25]. Studies using such models, though, have been frustrated by animal size, cost, and relative dearth of molecular reagents available. Recent work by Fang and colleagues [26], however, have established a mouse model of mandibular distraction osteogenesis to exploit the wide breadth of molecular reagents, microarray analysis, recent advances in bioluminescent reporting,

microcomputed tomography, and perhaps most importantly, transgene constructs available in mice. With the development of this model system, clear advantages arise with regard to cost, scalability, and flexibility for the performance of more detailed investigations to define the fundamental mechanisms behind successful bone deposition in the regenerate.

Mechanobiology

The impact of mechanical environment on bone development and maintenance is central to the study of mechanobiology. Dynamic loading has been shown to be critical for preservation and increase of bone mass in vivo and, on a cellular level, has been found to modulate osteoblast and osteoclast activity [27,28]. Recent studies also have suggested a role for hydrostatic stress and tensile strain in the orchestration of multipotent mesenchymal progenitor cell differentiation into bone, cartilage, and fibrous tissue [29–31]. In addition, cyclic motion and associated shear stress has been shown to accelerate cellular proliferation and callus production [29]. Nonetheless, as the significance of mechanical environment on bone formation is unmistakable, how forces contribute to proper osteogenesis in the distraction regenerate remains a paramount issue to be fully elucidated.

The characterization of resultant stress and strain patterns during distraction is essential to define how mechanical forces ultimately influence guided osteogenesis. By correlating histologic findings with measurements of tensile force, Loboa and colleagues [32] demonstrated the highest rates of bone formation occur during active mandibular distraction, with typical strain ranging between 10% and 12.5% across the regenerate. Measured strain was noted to have a viscoelastic response, reaching highest levels immediately following distraction and gradually declining to less than half the peak level with time [32]. Further work using finite element analysis revealed specific patterns of tensile strain and hydrostatic stress characteristics across the distraction gap. Within the gap itself, mesenchymal tissues were noted to experience moderate hydrostatic stress predictive of bone formation by way of intramembranous ossification. In contrast, mild compressive stress was observed in the periphery, compatible with endochondral ossification around the periosteal edges. These predictions based on finite element analysis remarkably mirror histologic findings in multiple animal models of mandibular distraction, with the appearance of direct bone formation within the distraction gap and cartilaginous intermediates adjacent to osteotomized fronts [26,32].

Having defined a blueprint for the stress engendered during distraction osteogenesis, recent investigations have focused on manipulations of the mechanical environment to accelerate successful bone deposition in the regenerate. Efforts to minimize the protracted course of standard protocols already have raised doubt over the necessity of a latency period, with recent studies demonstrating no significant benefit for delay of distraction [20, 33–35]. Investigations using the ovine and porcine models have exposed

no differences in mechanical strength, radiographic appearance, or bone density of the regenerate when latency periods of 4 or 7 days were compared with no latency [34,35]. Furthermore, retrospective studies have revealed similar results in the clinical setting, suggesting the traditional practice of latency–while still observed by most contemporary surgeons–may not be critically important and its reduction or elimination may serve to shorten the total duration of distraction osteogenesis without any detriment to bone deposition [20].

Though reduction of latency can afford a small gain in shortening the overall process of distraction, the greatest gains may conceivably be made by hastening the period of consolidation. Consequently, investigations have focused specifically on callus stimulation to accelerate maturation of the regenerate into mineralized bone. Axial loading in long bone fracture segments already has been shown to increase callus bulk, promote fracture healing, and hasten onset of bony union [36]. Adapting this principle to mandibular distraction, Mofid and colleagues [33] demonstrated cyclic loading of the regenerate during early consolidation to yield increased callus size, cortical density, and mineral apposition rate. Alternatively, callus stimulation has also been achieved through use of pulsed ultrasound, with analogous pro-osteogenic effects seen on bone formation in the distraction gap. The introduction of daily low-intensity ultrasound at a frequency of 1 kHz during mandibular distraction has been shown to accelerate time to completion of consolidation in rabbit models. Whether through cyclic loading or pulsed ultrasound, the notion of callus simulation therefore suggests an appealing approach to enhance bone formation and healing in craniofacial reconstruction. The application of callus stimulation to craniofacial distraction possibly may hasten the period of consolidation and thus minimize overall related patient morbidity. And by integrating this notion with data garnered from mechanical models of environmental stress, novel, more effective distraction protocols may be developed and translated into clinical practice.

Molecular biology

Although investigations have begun to elucidate the complex interplay of forces involved in bone formation during guided distraction, how this ultimately leads to changes at the cellular level to favor osteogenesis remains undefined. How cells respond to exogenous forces and translate these physical signals into a biomolecular cascade resides at the root of current investigations on mechanotransduction. Studies by Banes and colleagues [37] have established that forces can act directly at the cellular level, whether through mechanical-sensitive ion channels, integrin-cytoskeleton machinery, or load-sensitive receptor or nonreceptor tyrosine kinases. Furthermore, a link between the extracellular mechanical environment and intracellular signaling cascade recently has been demonstrated through the localization

of focal adhesion kinase protein to regions of bone formation during mandibular distraction [38]. As focal adhesion kinase has been implicated as an intermediary between cell-surface integrins and several MAP kinase cascades, a tangible biologic foundation for the influence of exogenous stress on bone formation has already been established.

With the advent of small animal models for mandibular distraction, significant strides have recently been made in defining the molecular processes regulating de novo bone formation in the regenerate. Studies have demonstrated a potential involvement for several pro-osteogenic cytokines, such as bone morphogenetic proteins (BMPs) and other members of the TGFβ superfamily [39–41]. Analyzing temporospatial expression patterns for BMPs 2, 4, and 7, histologic and immunohistochemical assessment have revealed an upregulation in osteoblasts during mandibular distraction [41]. Chondrocytes, likewise, were found to augment BMP expression particularly during the period of consolidation. Capitalizing on these findings, Ashinoff and colleagues [42] demonstrated that bone formation in the mandibular regenerate could be accelerated by local delivery of BMP-2 during consolidation through an adenoviral vector. By radiographic, histologic, and histomorphometric analyses, a significant increase in bone deposition could be induced, suggesting a biologic modality to enhance clinical distraction outcomes.

Although several investigations have highlighted the significance of BMPs in distraction osteogenesis, other cytokines have likewise gained increasing attention for their potential involvement in bone formation. Using a mouse model of mandibular distraction, Fang and colleagues [26] noted a dramatic rise in VEGF and FGF-2 expression during the period of active distraction. Quantitative real-time RT-PCR analysis revealed a fourfold increase in expression for both of these angiogenic factors relative to acutely lengthened hemimandible controls [26]. Immunohistochemical staining of goat mandibular regenerates have also demonstrated analogous findings, with intense staining for VEGF and FGF-2 during active distraction [43]. Recent studies designed to suppress these angiogenic signals have revealed provocative results, further underscoring the significance of an appropriate biomolecular environment for proper bone formation following mandibular distraction. Through administration of TNP-470, a fumagillin analog which inhibits endothelial cell proliferation and new capillary formation, complete nonunion was observed in all distracted hemimandibles [44–46]. Histologic assessment demonstrated no intramembranous bone formation within the distraction gap or evidence of endochondral bone along the periosteum. With PECAM staining showing no obvious blood vessel formation, the data suggest that direct failure of angiogenesis may, in part, have contributed to the failure of osteogenesis observed [46]. Despite appropriate mechanical signaling, an adequate angiogenic network–through VEGF or FGF-2 signaling–may thus be equally integral to the successful generation of new bone in the distraction gap.

Incorporating data obtained through mechanical investigations with recent findings in cytokine biology, a more lucid picture of the instruments guiding bone formation in distraction osteogenesis has therefore begun to develop. By using knowledge gained from mechanical stimulation and modeling of associated forces combined with manipulations in pro-osteogenic and pro-angiogenic cytokine signaling, a new paradigm for the clinical approach toward craniofacial distraction may emerge presently.

Cellular therapies

Despite the enormous potential for the generation of de novo bone using distraction osteogenesis, this modality nonetheless is limited in craniofacial repair. Some forms of craniosynostosis, certain craniofacial hypoplasias, and injuries secondary to facial trauma present clinical situations in which an approach using guided distraction may not engineer all of the necessary bone. The need for alternative modalities has therefore continued to drive the use of autogenous, allogeneic, and prosthetic materials to reconstruct the craniofacial skeleton [4,6,7,47–52]. As mentioned, however, these strategies are beset by numerous shortcomings, including infection, immunologic rejection, and graft-versus-host disease [51,53]. In addition, donor-site morbidity, in the case of autogenous bone harvest, may be protracted with ambulatory difficulty or chronic pain reported in as high as 51% of patients [54]. With these considerations in mind, researchers have therefore sought to develop novel methods to generate bone in the craniofacial skeleton.

Recent advances in cellular-based tissue engineering have made this a potentially attractive approach for the repair of bony defects given its widespread adaptability. With novel, moldable scaffolds providing specific molecular and environmental niches, the capacity for finely controlled bone formation is readily achievable. Contentious debate, however, has surrounded the identification of an optimal source for osteoprogenitors. Irrespective of this, the promise of tissue engineered bone through cell-based modalities has made this approach ever more appealing for the repair of calvarial and facial defects.

Cell-based approaches

Research has focused intently on defining the consummate cellular building block with which to base therapy for skeletal repair. Several human embryonic stem cell lines have been demonstrated to possess the capacity to differentiate into various tissue types [55]. Considerable controversy, however, has accompanied the study of these embryonic stem cells, with significant political and ethical hurdles encumbering further investigations [56–58]. Although work continues with somatic-cell nuclear transplantation for the generation of genotype predefined pluripotent cell-lines, the therapeutic use of such cells will continue to remain illusory in the foreseeable

future [59]. In similar fashion, recent debate has surrounded the clinical application of gene therapy and genetically modified adult cells [60]. Though early enthusiasm for this form of gene therapy has led to the race to develop treatments for genetic and non-genetic-based diseases, adverse outcomes have led to calls for a potential moratorium [60–63].

Over the last decade, the regenerative capacity of postnatal progenitor cells has increasingly emerged making these cells an attractive candidate for use in tissue-engineering applications. Whether these cells represent true pluripotent cells or more committed multipotent or oligopotent progenitors remains to be defined, but their capacity to differentiate into a multitude of cell types has been demonstrated abundantly [15,64–66]. Speculation, however, continues as to how these cells may function in tissue repair. Arguments for and against direct participation in the generation of new tissue or creation of conducive environments for endogenous host cell differentiation have been raised [67,68]. Nonetheless, the procurement and use of these postnatal progenitor cells allows for cellular based tissue engineering to proceed unfettered by the political and ethical concerns surrounding alternative cell sources.

Substantial work has already progressed with these postnatal progenitors, with early studies concentrating on mesenchymal stem cells (MSCs) naturally residing within bone marrow. Several investigators have demonstrated this cell population to contribute to the regeneration of other mesenchymal tissues throughout the body, including bone, cartilage, muscle, ligament, tendon, adipose, and stroma [15,69–71]. Furthermore, using bone marrow aspirates from over 350 human donors, Pittenger and colleagues [15] were able to show lineage specific differentiation of these MSCs into fat, cartilage, and bone under appropriate in vitro culture conditions. Not only did the human bone-marrow-derived MSCs demonstrate ability to extensively proliferate, but these cells also were capable of guided differentiation into multiple cell types, establishing a provocative cell source for potential craniofacial tissue engineering [15].

The concept of critical-sized defect reconstruction using mesenchymal stem cells harvested from bone marrow already has been validated in several animal models [72,73]. Implanting these cells within a fibrin glue construct into 15 mm parietal defects in rabbits, investigators have demonstrated healing and similar cellular integration into surrounding corticocancellous bone when compared with implanted osteoblasts [73]. Mechanical testing of the regenerate revealed equivalent stiffness and strength in defects filled with bone marrow-derived mesenchymal cells or harvested osteoblasts, both of which demonstrated significantly more healing than defects left unfilled [73]. Similar studies have found application of bone marrow-derived MSCs in the reconstruction of orbital defects in pigs [74]. But while great enthusiasm surrounds the use of these cells in craniofacial tissue engineering, several limiting factors have made bone marrow-derived MSCs less attractive. Selective sera and growth factor supplements have been reported by

some for ideal culture expansion before in vivo use [75–77]. In addition, as the estimated frequency of mesenchymal progenitor cells within the nucleated marrow cell fraction has been estimated to be as low as 1 in 27,000 cells, volumes of bone marrow aspirate larger than a few milliliters are frequently required [76,78]. Given the painful nature of this procedure, general or spinal anesthesia often may be necessary [76,79,80]. Finally, concerns related to donor-age associated changes in cellular biology have been raised frequently [81–84]. Therefore, while bone marrow-derived mesenchymal cells possess a potential for significant application in craniofacial bone engineering, there are current limitations associated with this cell source.

As an alternative to bone-marrow-derived mesenchymal cells, progenitor cells derived from the stromal fraction of adipose tissue recently have emerged as a potential cell source for craniofacial tissue engineering [66,85]. These adipose-derived mesenchymcal cells (AMCs), unlike their bone marrow counterpart, are more accessible and represent an available, readily expandable building block for the generation of bone [66,85]. In vitro studies of human AMC biology have demonstrated similar growth kinetics and cell senescence when compared with bone-marrow-derived cells obtained from the same donor [76]. In addition, no significant difference in gene transduction capacity was noted [76]. The real advantage, however, with AMCs reside in their ease for large volume procurement. Similar attempts with bone marrow harvest have yielded significant whole blood contamination such that the actual stem cell content can be compromised severely [86,87]. As procurement of adipose tissue is not subject to such contamination, larger amounts of adipose tissue can yield substantial numbers of potentially usable AMCs, while simultaneously avoiding more significant morbidity associated with bone marrow harvest [76,85].

Studies by Zuk and colleagues [66,85] have demonstrated the wide applicability of AMCs to the field of tissue engineering. Molecular and biochemical approaches using CD marker antigens have supported the notion that these cells are indeed multipotent stem cells capable of lineage-specific differentiation in the presence of precise induction factors [66]. In vitro studies using human AMCs have shown these cells to possess the ability to form fat, cartilage, muscle, and bone [85]. Similar investigations in mice have revealed an equally dynamic potential for these adipose-derived cells. Furthermore, under specific conditions, adult mouse-derived AMCs have been shown to retain similar osteogenic potential when compared with AMCs harvested from juvenile mice [88]. This highlights an additional advantage AMCs possess relative to their bone marrow counterparts, which have been shown to yield 41% fewer osteogenic progenitor cell colonies when harvested from older animals [81].

Several investigators have already established the utility of AMCs in bone tissue engineering. Seeding predifferentiated AMCs harvested from Lewis rats onto polyglycolic acid grafts. Lee and colleagues [89] demonstrated in vivo bone formation when implanting these constructs subcutaneously.

Immunohistochemistry and standard histologic staining suggested significantly increased bone deposition when compared with rats receiving unseeded grafts [89]. An equivalent capacity for human-derived AMCs seeded onto hydroxyapatite/tricalcium phosphate cubes to form bone in vivo also has been shown through subcutaneous implantation into SCID (severe combined immunodeficiency) mice [90]. With specific attention to craniofacial skeletal engineering, the authors' laboratory has shown the ability of mouse-derived AMCs, implanted into critical-sized calvarial defects on apatite-coated polylactic-coglycolic acid polymer scaffolds, to produce significant intramembranous bone formation by 2 weeks and complete bony bridging by 12 weeks as demonstrated radiographically [3]. While these investigations continue in animal models, the preliminary use of AMCs clinically already has been reported [9]. Using autologous adipose-derived stem cells combined with bone chips harvested from the iliac crest, surgeons in Germany were able to achieve bony repair of a large calvarial defect in a 7-year-old child; CT-scans 3 months postoperatively showed near complete calvarial continuity in the prior region of defect [9]. Though the true contribution of AMCs to the regenerate cannot be fully discerned from this case, results such as this engender boundless enthusiasm for the potential use of AMCs in craniofacial bone tissue engineering. In addition, these data suggest that AMCs alone may facilitate healing of critical-sized skeletal defects without the need for genetic manipulation.

Despite substantial progress made with these adipose-derived mesenchymal cells, however, significant gaps still exist in understanding their biology. Harvested cells represent a heterogeneous population comprised of not only mesenchymal cells but also pericytes, endothelial, and smooth muscle cells [85]. The potential implications of using this cellular mix clinically have yet to be determined. Furthermore, the population of mesenchymal cells alone may contain various subpopulations, each with different osteogenic potential. Future studies will endeavor to define these subpopulations and work toward the identification of the optimal cell fraction for use in the generation of bone. Nonetheless, the promise of mesenchymal cells for the repair of craniofacial skeletal defects remains attractive. With a readily available and cost-effective cell source in AMCs, a conceivable building block has been defined which may herald significant advances in craniofacial bone tissue engineering.

Bioengineered scaffolds

As research advances with multipotent progenitor cells, an increasing need for design of optimal biomimetic scaffolds has developed to facilitate cellular delivery for 3-dimensional tissue reconstruction. Fundamentally, these scaffolds should possess the capacity for osteoinduction, biocompatibility, and controlled biodegradation while maintaining structural integrity. In addition, ideal scaffolds would allow for delivery of signaling molecules

capable of coordinating cellular proliferation and differentiation. Considering the bulk of work already done with cellular-based bone engineering, a multitude of scaffolds already have been used, each with their own advantages and disadvantages [52,91–94]. Though several types have facilitated in vivo bone formation, the optimal scaffold has yet to be defined. Current scaffolds can be grouped broadly into three main categories: natural, mineral-based, and synthetic polymers.

Collagen and hyaluronic acid have been used routinely as substrates for bone engineering [91,94]. These natural scaffolds have been used in several craniofacial and dental applications, allowing for the generation of novel tissue [91,94]. Type I collagen has been used specifically to promote bone formation in rat mandibular defects [91]. Collagen alone placed into the fracture region led to histologic bone bridging following 6 weeks [91]. More recently, chitosan has emerged as another natural scaffold for use in craniofacial repair. A water-soluble form of chitin, chitosan has been shown to enhance healing of canine mandibular defects when injected into the regenerate [52]. Unfortunately, natural scaffolds often lack the desired structural rigidity for independent use in load-bearing regions. Therefore, their use in craniofacial reconstruction may be limited to areas with mechanical stability.

As an alternative to natural scaffolds, mineral-based scaffolds have been engineered to reproduce the molecular environment of bone. Composed of calcium phosphates in the form of hydroxyapatite or beta-tricalcium phosphate, these scaffolds have been used as early as 1920 [95]. By reproducing the three-dimensional structure of bone, these mineral lattices confer an osteoinductive signal to promote the maturation of progenitor cells down a path toward bone. Schleiphake and colleagues [96] have applied mineral-based scaffolds to the repair of calvarial defects in rats. Using several formulations of calcium phosphate, peri-implant bone deposition was noted around all scaffolds after 52 weeks [96]. By varying the content of calcium phosphate within the scaffolds, the rate of resorption also was noted to vary, reflecting a change in the biodegradability of the construct [96,97]. Like natural scaffolds, however, mineral-based scaffolds lack the strength for use in reconstruction of tissue subject to mechanical loads. Given their porous nature, these scaffolds can be brittle making fracture of the construct a common occurrence.

With the above limitations of available scaffolds, investigators have turned increasingly to synthetic scaffolds for their ability to be engineered with great durability. Polymers in use include polyglycolic acid, polylactic acid, polydioxanone, polycaprolactone, or various combinations of the above [98]. As knowledge and experience with these scaffolds increases, the potential for creation of a synthetic scaffold with precisely engineered rates of resorption has developed such that loss of mechanical strength can be controlled precisely [99]. But while synthetic scaffolds have the advantage for use in load-bearing regions, they typically lack the

osteoinductive properties of natural and mineral-based scaffolds. Current work has therefore focused on the generation of hybrid scaffolds possessing mineral and synthetic components. Studies by Kokubo and colleagues [100] have demonstrated the ability to coat polymer scaffolds with a uniform, dense, nano-crystalline apatite coating. These apatite-coated macroporous scaffolds combine the osteoconductve properties of apatites with the strength and versatility of degradable polymers. In vitro studies have already shown biomimetic apatites coated onto synthetic polymers to promote maturation of osteogenic precursors, with upregulation of osteocalcin and bone sialoprotein in MC3T3-E1 cells cultured on such surfaces [101]. Given these advances in scaffold design, the definition of an optimal niche for the engineering of novel bone is beginning to emerge. As the ultimate goal is to deliver osteogenic precursors in a conducive environment, however, future studies will look to develop new scaffolds that not only provide a strong lattice for mineralization but temporally and spatially control release of cytokines coordinating cellular proliferation and differentiation.

Summary

Craniofacial skeletal reconstruction represents a significant biomedical burden, with thousands of procedures performed annually to repair injuries and congenital malformations. The need for effective strategies to repair these bone deficits is apparent, but the multitude of approaches currently and historically used, highlight the need for development of novel strategies to engineer bone with minimal morbidity and in a cost-effective manner. Recent studies in distraction osteogenesis have begun to define the mechanical environment associated with successful bone deposition. Combined with knowledge gained from investigations on the biomolecular cascade within the distraction regenerate, a more effective application of distraction to the craniofacial skeleton will undoubtedly emerge. And in situations were this modality proves incapable or inappropriate for generating necessary bone, the promise of cellular-based tissue engineering using adipose-derived mesenchymal cells in concert with bioengineered scaffolds may provide for precise three-dimensional bone tissue reconstruction in the near future.

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