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INVITED MEDICAL REVIEW

Bioengineering strategies for regeneration of craniofacial bone: a review of emerging technologies

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Although advances in surgical techniques and bone grafting have significantly improved the functional and cosmetic restoration of craniofacial structures lost because of trauma or disease, there are still significant limitations in our ability to regenerate these tissues. The regeneration of oral and craniofacial tissues presents a formidable challenge that requires synthesis of basic science, clinical science, and engineering technology. Tissue engineering is an interdisciplinary field of study that addresses this challenge by applying the principles of engineering to biology and medicine toward the development of biological substitutes that restore, maintain, and improve normal function. This review will explore the impact of biomaterials design, stem cell biology and gene therapy on craniofacial tissue engineering.

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Introduction

The craniofacial unit is a complex array of bone, cartilage, soft tissue, nerves and vasculature; giving rise to the most esthetically important component of the body. Damage to these structures, even when minimal, usually leads to noticeable deformity. When changes are extensive, difficult reconstructive challenges result. In addition, in the case of tumor treatment, the craniofacial unit is frequently subjected to radiation and a variety of chemotherapeutic strategies, creating a compromised wound bed for reconstructive surgery.

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The bones of the craniofacial skeleton provide the foundation upon which other units are built and while soft tissue compensation is possible, adequate bony support is key to the return of both esthetics and function. For many years, bone grafts (autogenous, allogenic, and xenogenic) have been the mainstay for replacement. Current state of the art reconstruction of large bony maxillofacial defects involves free tissue transfer with microvascular reanastomosis of vascularized flaps from distant sites including fibula, iliac crest, scapula, and radius (Disa and Cordeiro, 2000; Emerick and Teknos, 2007). While these procedures have proven to be reliable and effective, they require extended hospitalization, and a secondary donor site with associated morbidity and complications. Furthermore, these techniques struggle to fully replicate normal form and function. As an alternative to the classic surgical approaches, developments in tissue engineering, gene therapy, and stem cell biology strive to utilize cells, biomaterial scaffolds and cell signaling factors to regenerate large skeletal defects with precise replication of normal body contours. A tissue engineering approach offers several potential benefits, including the lack of donor site morbidity, decrease in technical sensitivity of the repair, and most importantly, the ability to closely mimic the *in vivo* microenvironment in an attempt to recapitulate normal craniofacial development.

The optimal bone construct for repair would exactly replicate the lost structure, be fabricated with techniques generalizable to laboratories across the world, and would ultimately be replaced through the body's normal physiological processes of homeostasis over time. Furthermore, it would be reliable not only in standard tissue conditions but also in defects with compromised tissue beds following infection, radiation, chemotherapy and the scar of extensive trauma. Although no current technology meets all of these criteria, progress has been made in the preclinical arena that gives confidence to achieving this goal. The purpose of this review is to provide an overview and update on the current state of the art in bone reconstruction using new and evolving

technologies. Discussion will be focused on the broad categories of tissue engineering, gene therapy, and stem cell biology with attention to concepts and the current state of art based on ongoing research. While human clinical applications are limited to date, great promise exists as technology and our understanding of these principles increases over time.

Tissue engineering

Tissue engineering is an interdisciplinary field of study that applies the principles of engineering to biology and medicine toward the development of biological substitutes that restore, maintain, and improve normal function (Langer and Vacanti, 1993; Scheller *et al*, 2009). Cells, designed biomimetic materials, and biochemical signals compose the tissue engineering triad, and the significant interdependence of all three elements is often used in concert to produce constructs suitable for replacement of damaged or diseased tissue. The multidisciplinary nature of craniofacial tissue engineering strategies stems from formidable surgical challenges faced in the clinical setting, where the need to regenerate tissue that restores function to oral and craniofacial structures has long been of great importance (Hollinger and Winn, 1999; Alsberg *et al*, 2001; Steiner *et al*, 2002). Specifically, craniofacial bony structures such as the alveolar ridge (Hibi *et al*, 2006), the maxillary sinus floor (Shayesteh *et al*, 2008), and the hard palate (Carstens *et al*, 2005) have been reported to be successfully regenerated using tissue engineering strategies in clinical studies. Additionally, calvarial bone (Tu *et al*, 2007) and the mandibular condyle of the temporomandibular joint (TMJ) (Alhadlaq and Mao, 2005; Lee *et al*, 2009) have been reconstructed successfully in preclinical animal models. However, given the developmental differences between the appendicular skeleton (medosdermal origin – undergoes endochondral ossification) and the cranial skeleton (neural crest and paraxial mesodermal origin – undergoes both endochondral and intramembranous ossification), significant advances in understanding the complex developmental patterns of the craniofacial unit must take place to reach the goal of using optimally designed biomaterials for human clinical applications (Jiang *et al*, 2002). Therefore, the engineering design principles and techniques needed to achieve the goal of controlling and regenerating components of an intricate biological tissue system such as the oral and craniofacial complex are diverse. The focus of this review will be to describe successful models currently applying these principles in the preclinical and clinical setting, although it is important to note that the preclinical findings may not always translate to the human experience.

In the bone tissue engineering paradigm, the purpose of the scaffold is to provide an osteoinductive extracellular matrix (ECM) analog to support initial cell adhesion, growth and development of new bone. Within the craniofacial tissue engineering field, the major classes of materials used are natural polymers, synthetic polymers, ceramics, composite materials, and electrospun nanofibers. In addition to selection of the appropriate material, the scaffold should possess design characteristics that elicit a biological response from the microenvironment. Through optimizing design parameters and fabrication techniques, the ideal scaffold candidate is required to achieve the following: (i) to deliver progenitor cells or facilitate host cell recruitment via osteoconductive and osteoinductive material properties; (ii) to deliver important signaling molecules in a temporally and spatially controlled manner via growth factor incorporation and surface modification; (iii) to promote vascularization and tissue in-growth via changes in microporosity; (iv) to properly fit the shape of the anatomical defect via image and Computer-Aided Design (CAD) based scaffold design methods; (v) to provide initial plasticity while maintaining load-bearing stability via selection of the appropriate fabrication technique; and (vi) to degrade into biocompatible byproducts at a rate that matches new tissue formation via selection of the optimal material composition (Giesen *et al*, 2004; Meinel *et al*, 2004; Hollister, 2005; Mao *et al*, 2006; Gersbach *et al*, 2007; Hutmacher and Cool, 2007; Hutmacher *et al*, 2007; Wang *et al*, 2007; Jones *et al*, 2009). To address the requirements of scaffolds specifically for craniofacial bone repair, one must consider the delicate balance of imparting sufficient porosity, permeability, and pore architecture to facilitate vascular invasion, mass transport, and cell survival in the scaffold interior, while maintaining the ability to withstand load bearing conditions that craniofacial bone sustains as a result of oral functions such as mastication, deglutition, and speech (Hollister, 2005; Hutmacher and Cool, 2007; Jones *et al*, 2007; Potier *et al*, 2007).

The requirement to include more of the above design criteria into a single biomaterial construct has spurred extensive investigation on the use of natural, synthetic, and composite materials. Natural scaffolds such as collagen type I, chitosan, calcium alginate, hyaluronic acid and composites have been shown to be osteoconductive, thus suitable for bone formation both *in vitro* and *in vivo* (Solchaga *et al*, 2002; Abbah *et al*, 2006; Chang *et al*, 2009; Chesnutt *et al*, 2009). However, a major disadvantage is the lack of mechanical integrity. Alternatives with structural stability have been investigated, including the use of synthentic polymers such as poly(lactic acid) (PLA), poly(glycolic acid) PGA, co-polymer polylactic-co-glycolic acid (PLGA), poly(methyl methacrylate) (PMMA) and poly(caprolactone) (PCL). These polymers exhibit the ability to support osteoblastic differentiation and bone tissue formation, and are therefore commonly used for oral and maxillofacial applications (Li *et al*, 2005; Petrie *et al*, 2008; Kretlow *et al*, 2009). Additionally, polymer/ceramic composite materials such as PLLA/HA have been used successfully as ostechondral constructs for TMJ engineering via the delivery of porcine chondrocytes in the polymer layer and fibroblasts transduced with adenovirus driving the expression of bone morphogenetic proteins (BMPs) (Schek *et al*, 2004, 2005).

Designing matrices suitable for the recruitment of osteoprogenitor/stem cells has been promoted by the approach of mimicking the composition, morphological

traits and mechanical function of the native bone ECM through the use of a wide range of electrospun bone regenerative nanofiber materials such as PLLA, PGA, PCL, silk fibroin, calcium phosphates, bioactive glass and glass ceramics (Jang *et al*, 2009). The nanofibrous structure is becoming increasingly useful and popular within the field of craniofacial tissue engineering because these materials are able to assist cells along an osteogenic pathway via nanoscale organization and novel nanocomposite systems (Kim *et al*, 2006).

The addition of osteoinductive factors is also essential to the success of craniofacial bone regeneration strategies. BMPs, primarily 2, 4 and 7, fulfill this role in the vast majority of applications. BMPs are a family of growth factors important to bone formation and regeneration which have been studied extensively (Reddi, 2005) and are now available in a recombinant form for human clinical application. One of the limiting factors in this methodology has been the duration of the BMP's presence at the site of action with one of the potential roles of the scaffolds being to assist in controlling growth factor release. Importantly, the balance of sustained release required for optimal effect while avoiding overly high concentrations leading to potential untoward effects must be managed.

Several preclinical models exist for the use of BMPs and recombinant human BMP (rhBMP) as a concentrate delivered on scaffolds, composites, and hydrogels. Defects studied have included animal models for ulna, radius, femur, alveolar cleft, spine, orbital floor, tibia, and calvarial defects (Bessa *et al*, 2008). At present, the only FDA-approved craniofacial application of rhBMP is for sinus augmentation. A recent report highlighting this use enrolled 160 subjects in a multi-center, randomized prospective trial using rhBMP on a collagen sponge versus autogenous bone graft for two-stage maxillary sinus floor augmentation. Outcomes measured included new bone formation, implant integration and functional loading at 6 months and 2 years. Success rates for both groups was 79% with significantly denser bone in the rhBMP-2 group. In addition, the autograft group was noted to have 17% long-term parasthesia, pain, or gait disturbance, highlighting the effects of donor site morbidity.

Limited clinical studies exist for tissue engineering concepts other than BMPs and platelet-derived growth factors (PDGF) in any body location. The majority of evidence is derived from small case series without controls and pertains to general orthopedic, rather than craniofacial applications. In one study, three patients with aneurysmal bone cyst, giant cell tumor, and fibrous dysplasia were treated with implants based on HA ceramics with multipotent stem cells induced toward the osteoblast lineage. Successful healing was reported although only based on plain radiographs and computerized tomography. There were no adverse reactions noted in this study with a limited number of patients (Morishita *et al*, 2006).

The preclinical and clinical studies reviewed here indicate that significant advancements are being made in the design of biomaterials to create replacements for damaged or pathological tissues. As biomaterials become highly functionalized platforms upon which hybrid tissues can develop, craniofacial tissue engineering strategies hold significant promise for future clinical use.

Stem cell biology

From an embryological perspective, the structures of the craniofacial region are predominantly derived from cells of neural crest mesenchymal origin. These cells, together with their synergistic partners, mesodermal cells, both arise from the embryonic stem cell (Couly *et al*, 1993; Jiang *et al*, 2002). Mesenchymal stem cells (MSCs) are adult cells from this lineage that persist with the ability to repair and regenerate tissue following insult. MSCs have the genetic capacity to differentiate into both mesenchymal and bone tissue lineage making them a useful tool and target for research in tissue regeneration (Krebsbach *et al*, 1997, 1999). Highlighting this potential is the fact that a single population of MSCs can differentiate into both chondrocytes and osteoblasts based on external factors to produce both bone and cartilage. MSCs are self renewable lineages which were first isolated and reported nearly four decades ago, and since then, techniques for isolation have become increasingly effective in regard to throughput and specificity with current use of fluorescence-activated, magnetic-activated cell-sorting, and genomic profiling (Friedenstein *et al*, 1966; Gronthos *et al*, 1999; Jones *et al*, 2002; Kinnaird *et al*, 2004). In addition, cloning technology can be harnessed to create large quantities of the desired cells. Given their properties, stem cells have been utilized in a variety of preclinical applications for regeneration of craniofacial bone.

One example of a complex bioengineering strategy for craniofacial bone, highlighting its potential, is the development of the mandibular condyle. It has been shown that the generation of (i) a cadaveric human mandibular condyle of bone and cartilage derived from adult rat bone marrow MSCs, exposed to osteogenic and chondrogenic conditions, and encapsulated in poly(ethylene glycol) diacrylate (PEDGA) hydrogels (Alhadlaq and Mao, 2003), and (ii) the vascularization and ectopic tissue formation of an anatomically shaped human tibia condyle made of a composite poly-ϵ-caprolactone and hydroxyapatite material seeded with osteogenically differentiated human MSCs after 6 weeks of subcutaneous implantation in athymic rats (Lee *et al*, 2009), are possible. This suggests that optimal cell types and local factors are required to make the possibility of an engineered condyle look promising for human application.

Adipose derived stem cells (ADSCs) are an alternative progenitor cell source, in that these cells are easily accessible through lipo aspirate and can be expanded *in vitro* to form adipocytes, chondrocytes and osteoblasts (Zuk *et al*, 2001). Additionally, ADSCs have been successfully applied to scaffolds and have undergone *de novo* formation of bone in subcutaneous tissue, calvarial defects, and critical sized bone defects in murine models (Lee *et al*, 2003; Yoon *et al*, 2007; Hao

et al, 2008). Translation of this technology to human application has been reported with ADSCs combined with iliac crest bone graft for a calvarial bone defect (Zuk *et al*, 2001; Shi *et al*, 2005).

In addition to MSCs and ADSCs, there has been extensive investigation into the capability of MSCs derived from various craniofacial tissues that form bone-like, dentin-like, and cementum-like structures. For instance, dental pulp stem cells have been isolated from dental pulp and in response to osteogenic conditions, have been shown to express osteogenic and odontogenic markers and form mineral deposits on dentin-like structures (Gronthos *et al*, 2000; Murray *et al*, 2001; Ueno *et al*, 2001). Periodontal ligament stem cells (PDLSCs) first isolated, sorted, and cultured from extracted third molars of human origin have been found to differentiate into adipocytes and osteoblasts *in vitro*, and generate cementum-like structures interfaced with dense collagen fibers *in vivo* (Shi *et al*, 2002; Seo *et al*, 2004). Lastly, *in vitro* expansion of human exfoliated deciduous teeth stem cells have been shown to have proliferative capacity, a surface antigen profile similar to MSCs, and have the ability to express osteogenic genes and form dentin-like structures *in vivo* when seeded onto HA/TCP scaffolds (Miura *et al*, 2003).

Although the cell types discussed have potential for great use for bone repair, given the complex nature of craniofacial bone, the use of human embryonic stem cells (hESCs) might provide a repository of cells that can be isolated, manipulated, and utilized for future cell-based engineering strategies (Thomson *et al*, 1998). One goal for hESC research is the controlled differentiation into specific progenitor cells for the purpose of replacing or regenerating damaged tissue. Therefore, the ability to obtain large quantities of multipotent cells from hESCs represents a challenge for cell based therapy and tissue engineering strategies that currently rely on human bone marrow stromal cells (hBMSCs). Recent studies show that mesenchymal precursors have been derived from hESCs (hES-MSCs) via various isolation methods, and the generation of osteoblasts has been achieved in the presence of known osteogenic supplements and co-culture with primary bone derived cells (Sotille *et al*, 2003; Bielby *et al*, 2004; Barberi *et al*, 2005; Cao *et al*, 2005; Ahn *et al*, 2006, Karp *et al*, 2006; Olivier *et al*, 2006; Duplomb *et al*, 2007; Karner *et al*, 2007; Tong *et al*, 2007; Arpornmaeklong *et al*, 2009; Brown *et al*, 2009). However, the identification and characterization of a pure osteoprogenitor cell population has yet to be achieved. Osteoprogenitor cells derived from hESCs have tremendous potential, as they can serve as a tool through which one can not only characterize early bone development and cellular behavior on bone-related biomaterials but also have application specifically in craniofacial bone repair.

To enhance the understanding of the differentiation pattern and bone formation capacity of hESCs in the skeletal defect, investigators have studied the complete temporal pattern of osteoblastic differentiation of hES-MSCs in a long-term culture, as well as the influence of the three-dimensional matrix on the osteogenic differentiation and bone formation capacity of hES-MSCs in the calvarial defect. It was found that incubation of hES-MSCs in osteogenic medium induced osteoblastic differentiation in a similar chronological pattern to previously reported human bone marrow stromal cells (hBMSCs) and primary osteoblasts. Furthermore, it was also demonstrated that differentiation was enhanced by three-dimensional matrix of collagen scaffolds (Arpornmaeklong *et al*, 2009). The fate of transplanted cells in the bone formation process was verified by identifying the presence of human cells in the matrix of the newly formed bone, suggesting that hES-MSCs represent an osteoprogenitor population that can be sorted, enriched and manipulated for use in craniofacial engineering strategies. Of particular importance is the fact that the lineage progression through both mesodermal and neural crest lineages can be controlled for hESCs, addressing one of the major challenges unique to healing the cranial skeletal given the dual embryonic origins (Jiang *et al*, 2002; Mao *et al*, 2006; Lian *et al*, 2007; Brown *et al*, 2009; Goldstein *et al*, 2010).

Gene therapy

Gene therapy depends on the transfer of genetic material into living cells for the purposes of treating a disease process or regenerating tissues. Both viral and non-viral vectors for gene transfer have been developed and applied to pre-clinical and limited clinical settings (Scheller and Krebsbach, 2009). Non-viral vectors have advantages attributable to safety and the ability to introduce large segments of DNA; however, viral vectors remain the mainstay of therapy as a result of their efficacy in transmitting the genetic material to the host. Compared with the focus of gene therapy on treatment of disease, the use of gene therapy for the regeneration of craniofacial bone has been somewhat limited. However, animal craniofacial models for gene therapy do exist not only for bone (Krebsbach *et al*, 2000; Dunn *et al*, 2005), but also for cartilage (Palmer *et al*, 2002) and periodontal ligament (Jin *et al*, 2004) as well as complex multiunit structures such as the TMJ (Nakashima *et al*, 2006; Rabie *et al*, 2007) and salivary glands (Cotrim *et al*, 2006; Voutetakis *et al*, 2008).

A number of applications are currently evolving with promise for future augmentation of the bone healing process or replacement of missing bone. BMPs have maintained a prominent role in this model with potential advantages of gene therapy conveying the ability to maintain protein expression at clinically effective levels over longer periods of time compared with direct delivery of protein. In addition, because of the sustained presence, gene delivery techniques are one of the few modalities studied for potential application in irradiated fields (Nussenbaum *et al*, 2003; Hu *et al*, 2010).

Enhanced fracture repair and bone formation have been achieved using viral-mediated gene delivery. A number of studies (Peng *et al*, 2004a; Shen *et al*, 2004a,b) using muscle-derived cells for ex vivo transduction of bone resulted in fracture repair when placed into femur critical sized defects. Transduction with

BMP-4 leads to radiographic and histological healing with bone, which was 77 ± 28% the strength of normal control femur. Other work has demonstrated that the addition of VEGF to BMP-4 enhances this process. Likewise, other groups have reported a number of studies using a similar approach with BMP-2 (Betz *et al*, 2010). In this model, an expedited ex vivo approach where muscle or fat fragments were transduced with BMP-2 and incubated in tissue culture only 24 h prior to implantation. Moreover, the femoral critical size defect model was used with radiographic and histological evaluation. Bone volumes in the transduced sites showed statistically significant increases in bone formation with 100% of bone defects being bridged in the study animals. BMPs 6, 7 and 9 have also been utilized in gene therapy bone repair models (Alden *et al*, 2000; Jane *et al*, 2002) with similar abilities to induce bone formation.

While BMPs have been the most frequently investigated factors for gene delivery, alternatives have been described. Sonic hedgehog (Shh), PDGF-B, and constitutively active activin-like kinase 2 (caALK2) have all been investigated. Shh was studied in a craniofacial model using rabbit cranial defects (Edwards 2005). Human Shh cDNA was isolated from fetal lung tissue and cloned into the replication-incompetent retroviral expression vector LNCX. Alginate/type I collagen constructs with Shh transduced cells were placed in cranial defects and examined grossly, radiographically, and histologically at 6 weeks. Shh treated animals had statistically significant bone healing compared with controls and no treatment related side effects were noted. Interestingly, caALK2 has been investigated using an allograft model for enhanced healing. Allografts were coated with AAV-caALK2 vector which mediated *in vivo* gene transfer. The AAV vector was capable of transducing osteoblasts in the fracture callus and directly inducing bone formation (Anusaksathien *et al*, 2004; Ulrich-Vinther, 2007).

In an effort to determine if regenerative gene therapy methods could overcome the negative effects of radiation therapy, animal models that more directly mimic the compromised wound beds of irradiated cancer patients have been used for testing *ex vivo* (Nussenbaum *et al*, 2003) and *in vivo* (Hu *et al*, 2010) gene therapy. In a critical size calvarial bone defect animal model, bone defects were treated with an inlay calvarial bone graft or transduced dermal fibroblasts. Two weeks postoperatively animals were randomized to receive 12-Gy radiation or no radiation control. While radiation treatment decreased the amount of bone formation from 87% to 65% bone regeneration was still successful giving promise to the possibilities of treating ablative defects prior to radiation therapy with these treatment modalities (Nussenbaum *et al*, 2003).

An alternative approach to gain more control over the timing of expression of transferred genes has been the use of inducible vector systems. An example of this is the doxycycline inducible 'tetON' promoter system with selective induction of BMP-2 or BMP-4. In bone regeneration models, only vector containing sites induced bone following administration of oral doxycycline (Gafni *et al*, 2004; Peng *et al*, 2004b). Taken together, these examples illustrate the potential benefits of gene therapy over other bioengineering approaches and will undoubtedly continue its progress as a leading candidate for future clinical applications.

Conclusion

Craniofacial tissue engineering research has tremendously developed over the last two decades as a result of the individual strides made in the fields of developmental biology, stem cell biology, polymer chemistry, mechanical engineering, and biomedical engineering. The unique challenges faced by maxillofacial surgeons in the clinical setting has spurred investigation of effective tissue engineering strategies that involve isolated and enriched progenitor cells, sophisticated gene delivery methods, and complex biomaterial scaffold fabrication and design techniques. As we begin to understand how biomaterial properties influence cellular behavior, we will progress toward the development of biomimetic scaffolds that contain incorporated signaling cues that induce cellular differentiation and ECM deposition, possess composite material properties that have the ability to generate hybrid tissue, and have tunable three-dimensional geometrical architecture that appropriately restores form and function to craniofacial bone defects.

References

Abbah SA, Lu WW, Chan D *et al* (2006). In vitro evaluation of alginate encapsulated adipose-tissue stromal cells for use as injectable bone graft substitute. *Biochem Biophys Res Commun* **347:** 185–191.

Ahn S, Kim S, Park K *et al* (2006). Primary bone-derived cells induce osteogenic differentiation without exogenous factors in human embryonic stem cells. *Biochem Biophys Res Commun* **340:** 403–408.

Alden TD, Beres EJ, Laurent JS *et al* (2000). The use of bone morphogenetic protein gene therapy in craniofacial bone repair. *J Craniofac Surg* **11:** 24–30.

Alhadlaq A, Mao JJ (2003). Tissue-engineered neogenesis of human-shaped mandibular condyle from rat mesenchymal stem cells. *J Dent Res* **82:** 951–956.

Alhadlaq A, Mao JJ (2005). Tissue-engineered osteochondral constructs in the shape of an articular condyle. *J Bone Joint Surg Am* **87:** 936–944.

Alsberg E, Hill E, Mooney D (2001). Craniofacial tissue engineering. *Crit Rev Oral Biol Med* **12:** 64–75.

Anusaksathien O, Jin Q, Zhao M, Somerman MJ, Giannobile WV (2004). Effect of sustained gene delivery of platelet-derived growth factor or its antagonist (PDGF-1308) on tissue-engineered cementum. *J Periodontol* **75:** 429–440.

Arpornmaeklong P, Brown SE, Wang Z, Krebsbach PH (2009). Phenotypic characterization, osteoblastic differentiation, and bone regeneration capacity of human embryonic stem cell-derived mesenchymal stem cells. *Stem Cells Dev* **18:** 955–968.

Barberi T, Willis LM, Socci ND, Studer L (2005). Derivation of multipotent mesenchymal precursors from human embryonic stem cells. *PLoS Med* **2:** e161.

Bessa PC, Casal M, Reis RL (2008). Bone morphogenetic proteins in tissue engineering: the road from laboratory to clinic, part II (BMP delivery). *J Tissue Eng Regen Med* **2:** 81–96.

Betz OB, Betz VM, Abdulazim A *et al* (2010).The repair of critical size bone defects using expedited, autologous BMP-2 gene activated fat implants. *Tissue Eng Part A* **16:** 1093–1101.

Bielby B, Boccaccini A, Polak J, Buttery L (2004). In vitro differentiation and in vivo mineralization of osteogenic cells derived from human embryonic stem cells. *Tissue Eng* **10:** 1518–1525.

Brown SE, Tong W, Krebsbach PH (2009). The derivation of mesenchymal stem cells from human embryonic stem cells. *Cells Tissues Organs* **189:** 256–260.

Cao T, Heng B, Ye C *et al* (2005). Osteogenic differentiation within intact human embryoid bodies result in a marked increase in osteocalcin secretion after 12 days of in vitro culture, and formation of morphologically distinct nodule-like structures. *Tissue Cell* **37:** 325–334.

Carstens MH, Chin M, Ng T, Tom WK (2005). Reconstruction of #7 facial cleft with distraction-assisted in situ osteogenesis (DISO): role of recombinant human bone morphogenetic protein-2 with Helistat-activated collagen implant. *J Craniofac Surg* **16:** 1023–1032.

Chang SH, Hsu YM, Wang YJ, Tsao YP, Tung KY, Wang TY (2009). Fabrication of pre-determined shape of bone segment with collagen-hydroxyapatite scaffold and autogenous platelet-rich plasma. *J Mater Sci Mater Med* **20:** 23–31.

Chesnutt BM, Yuan Y, Buddington K, Haggard WO, Bumgardner JD (2009). Composite chitosan/nano-hydroxyapatite scaffolds induce osteocalcin production by osteoblasts in vitro and support bone formation in vivo. *Tissue Eng Part A* **15:** 2571–2579.

Cotrim AP, Mineshiba F, Sugito T, Samuni Y, Baum BJ (2006). Salivary gland gene therapy. *Dent Clin North Am* **50:** 157–173, vii.

Couly GF, Coltey PM, Le Douarin NM (1993). The triple origin of skull in higher vertebrates: a study in quail-chick chimeras. *Development* **117:** 409–429.

Disa JJ, Cordeiro PG (2000). Mandible reconstruction with microvascular surgery. *Semin Surg Oncol* **19:** 226–234.

Dunn CA, Jin Q, Taba M Jr, Franceschi RT, Bruce Rutherford R, Giannobile WV (2005). BMP gene delivery for alveolar bone engineering at dental implant defects. *Mol Ther* **11:** 294–299.

Duplomb L, Dagouassat M, Jourdon P, Heymann D (2007). Concise review: Embryonic stem cells: a new tool to study osteoblast and osteoclast differentiation. *Stem Cells* **25:** 544–552.

Edwards PC, Ruggiero S, Fantasia J *et al* (2005). Sonic hedgehog gene-enhanced tissue engineering for bone regeneration. *Gene Ther* **12:** 75–86.

Emerick KS, Teknos TN (2007). State-of-the-art mandible reconstruction using revascularized free-tissue transfer. *Expert Rev Anticancer Ther* **7:** 1781–1788.

Friedenstein AJ, Piatetzky S II, Petrakova KV (1966). Osteogenesis in transplants of bone marrow cells. *J Embryol Exp Morphol* **16:** 381–390.

Gafni Y, Pelled G, Zilberman Y *et al* (2004). Gene therapy platform for bone regeneration using an exogenously regulated, AAV-2-based gene expression system. *Mol Ther* **9:** 587–595.

Gersbach CA, Coyer SR, Le Doux JM, Garcia AJ (2007). Biomaterial-mediated retroviral gene transfer using self-assembled monolayers. *Biomaterials* **28:** 5121–5127.

Giesen EB, Ding M, Dalstra M, van Eijden TM (2004). Changed morphology and mechanical properties of cancellous bone in the mandibular condyles of edentate people. *J Dent Res* **83:** 255–259.

Goldstein RS, Pomp O, Brokhman I, Ziegler L (2010). Generation of neural crest cells and peripheral sensory neurons from human embryonic stem cells. *Methods Mol Biol* **584:** 283–300.

Gronthos S, Zannettino AC, Graves SE, Ohta S, Hay SJ, Simmons PJ (1999). Differential cell surface expression of the STRO-1 and alkaline phosphatase antigens on discrete developmental stages in primary cultures of human bone cells. *J Bone Miner Res* **14:** 47–56.

Gronthos S, Mankani M, Brahim J, Robey PG, Shi S (2000). Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. *Proc Natl Acad Sci USA* **97:** 13625–13630.

Hao W, Hu YY, Wei YY *et al* (2008). Collagen I gel can facilitate homogenous bone formation of adipose-derived stem cells in PLGA-beta-TCP scaffold. *Cells Tissues Organs* **187:** 89–102.

Hibi H, Yamada Y, Ueda M, Endo Y (2006). Alveolar cleft osteoplasty using tissue-engineered osteogenic material. *Int J Oral Maxillofac Surg* **35:** 551–555.

Hollinger J, Winn S (1999). Tissue engineering of bone in the craniofacial complex. *Ann NY Acad Sci* **875:** 379–385.

Hollister SJ (2005). Porous scaffold design for tissue engineering. *Nat Mater* **4:** 518–524.

Hu WW, Ward BB, Wang Z, Krebsbach PH (2010). Bone regeneration in defects compromised by radiotherapy. *J Dent Res* **89:** 77–81.

Hutmacher DW, Cool S (2007). Concepts of scaffold-based tissue engineering – the rationale to use solid free-form fabrication techniques. *J Cell Mol Med* **11:** 654–669.

Hutmacher DW, Schantz JT, Lam CX, Tan KC, Lim TC (2007). State of the art and future directions of scaffold-based bone engineering from a biomaterials perspective. *J Tissue Eng Regen Med* **1:** 245–260.

Jane JA Jr, Dunford BA, Kron A *et al* (2002). Ectopic osteogenesis using adenoviral bone morphogenetic protein (BMP)-4 and BMP-6 gene transfer. *Mol Ther* **6:** 464–470.

Jang JH, Castano O, Kim HW (2009). Electrospun materials as potential platforms for bone tissue engineering. *Adv Drug Deliv Rev* **61:** 1065–1083.

Jiang X, Iseki S, Maxson RE, Sucov HM, Morriss-Kay GM (2002). Tissue origins and interactions in the mammalian skull vault. *Dev Biol* **241:** 106–116.

Jin Q, Anusaksathien O, Webb SA, Printz MA, Giannobile WV (2004). Engineering of tooth-supporting structures by delivery of PDGF gene therapy vectors. *Mol Ther* **9:** 519–526.

Jones EA, Kinsey SE, English A *et al* (2002). Isolation and characterization of bone marrow multipotential mesenchymal progenitor cells. *Arthritis Rheum* **46:** 3349–3360.

Jones AC, Arns CH, Sheppard AP, Hutmacher DW, Milthorpe BK, Knackstedt MA (2007). Assessment of bone ingrowth into porous biomaterials using MICRO-CT. *Biomaterials* **28:** 2491–2504.

Jones AC, Arns CH, Hutmacher DW, Milthorpe BK, Sheppard AP, Knackstedt MA (2009). The correlation of pore morphology, interconnectivity and physical properties of 3D ceramic scaffolds with bone ingrowth. *Biomaterials* **30:** 1440–1451.

Karner E, Unger C, Sloan AJ, Richter-Ahrlund L, Sugars R, Wendel M (2007). Bone matrix formation in osteogenic cultures derived from human embryonic stem cells in vitro. *Stem Cells Dev* **16:** 39–52.

Karp J, Ferreira L, Khademhosseini A, Kwon A, Yeh J, Langer R (2006). Cultivation of human embryonic stem cells without the embryoid body step enhances osteogenesis in vitro. *Stem Cells* **24:** 835–843.

Kim HW, Song JH, Kim HE (2006). Bioactive glass nanofiber-collagen nanocomposite as a novel bone regeneration matrix. *J Biomed Mater Res A* **79:** 698–705.

Kinnaird T, Stabile E, Burnett MS *et al* (2004). Local delivery of marrow-derived stromal cells augments collateral perfusion through paracrine mechanisms. *Circulation* **109:** 1543–1549.

Krebsbach PH, Kuznetsov SA, Satomura K, Emmons RV, Rowe DW, Robey PG (1997). Bone formation in vivo: comparison of osteogenesis by transplanted mouse and human marrow stromal fibroblasts. *Transplantation* **63:** 1059–1069.

Krebsbach PH, Kuznetsov SA, Bianco P, Robey PG (1999). Bone marrow stromal cells: characterization and clinical application. *Crit Rev Oral Biol Med* **10:** 165–181.

Krebsbach PH, Gu K, Franceschi RT, Rutherford RB (2000). Gene therapy-directed osteogenesis: BMP-7-transduced human fibroblasts form bone in vivo. *Hum Gene Ther* **11:** 1201–1210.

Kretlow JD, Young S, Klouda L, Wong M, Mikos AG (2009). Injectable biomaterials for regenerating complex craniofacial tissues. *Adv Mater Deerfield* **21:** 3368–3393.

Langer R, Vacanti JP (1993). Tissue engineering. *Science* **260:** 920–926.

Lee JA, Parrett BM, Conejero JA *et al* (2003). Biological alchemy: engineering bone and fat from fat-derived stem cells. *Ann Plast Surg* **50:** 610–617.

Lee CH, Marion NW, Hollister S, Mao JJ (2009). Tissue formation and vascularization in anatomically shaped human joint condyle ectopically in vivo. *Tissue Eng Part A* **15:** 3923–3930.

Li WJ, Tuli R, Huang X, Laquerriere P, Tuan RS (2005). Multilineage differentiation of human mesenchymal stem cells in a three-dimensional nanofibrous scaffold. *Biomaterials* **26:** 5158–5166.

Lian Q, Lye E, Suan Yeo K *et al* (2007). Derivation of clinically compliant MSCs from CD105+, CD24- differentiated human ESCs. *Stem Cells* **25:** 425–436.

Mao JJ, Giannobile WV, Helms JA *et al* (2006). Craniofacial tissue engineering by stem cells. *J Dent Res* **85:** 966–979.

Meinel L, Karageorgiou V, Hofmann S *et al* (2004). Engineering bone-like tissue in vitro using human bone marrow stem cells and silk scaffolds. *J Biomed Mater Res A* **71:** 25–34.

Miura M, Gronthos S, Zhao M *et al* (2003). SHED: stem cells from human exfoliated deciduous teeth. *Proc Natl Acad Sci USA* **100:** 5807–5812.

Morishita T, Honoki K, Ohgushi H, Kotobuki N, Matsushima A, Takakura Y (2006). Tissue engineering approach to the treatment of bone tumors: three cases of cultured bone grafts derived from patients' mesenchymal stem cells. *Artif Organs* **30:** 115–118.

Murray PE, About I, Franquin JC, Remusat M, Smith AJ (2001). Restorative pulpal and repair responses. *J Am Dent Assoc* **132:** 482–491.

Nakashima M, Iohara K, Zheng L (2006). Gene therapy for dentin regeneration with bone morphogenetic proteins. *Curr Gene Ther* **6:** 551–560.

Nussenbaum B, Rutherford RB, Teknos TN, Dornfeld KJ, Krebsbach PH (2003). Ex vivo gene therapy for skeletal regeneration in cranial defects compromised by postoperative radiotherapy. *Hum Gene Ther* **14:** 1107–1115.

Olivier EN, Rybicki AC, Bouhassira EE (2006). Differentiation of human embryonic stem cells in bipotent mesenchymal stem cells. *Stem Cells* **24:** 1914–1922.

Palmer G, Pascher A, Gouze E *et al* (2002). Development of gene-based therapies for cartilage repair. *Crit Rev Eukaryot Gene Expr* **12:** 259–273.

Peng H, Usas A, Gearhart B, Olshanski A, Shen HC, Huard J (2004a). Converse relationship between in vitro osteogenic differentiation and in vivo bone healing elicited by different populations of muscle-derived cells genetically engineered to express BMP4. *J Bone Miner Res* **19:** 630–641.

Peng H, Usas A, Gearhart B, Young B, Olshanski A, Huard J (2004b). Development of a self-inactivating tet-on retroviral vector expressing bone morphogenetic protein 4 to achieve regulated bone formation. *Mol Ther* **9:** 885–894.

Petrie C, Tholpady S, Ogle R, Botchwey E (2008). Proliferative capacity and osteogenic potential of novel dura mater stem cells on poly-lactic-co-glycolic acid. *J Biomed Mater Res A* **85:** 61–71.

Potier E, Ferreira E, Andriamanalijaona R *et al* (2007). Hypoxia affects mesenchymal stromal cell osteogenic differentiation and angiogenic factor expression. *Bone* **40:** 1078–1087.

Rabie AB, Dai J, Xu R (2007). Recombinant AAV-mediated VEGF gene therapy induces mandibular condylar growth. *Gene Ther* **14:** 972–980.

Reddi AH (2005). BMPs: from bone morphogenetic proteins to body morphogenetic proteins. *Cytokine Growth Factor Rev* **16:** 249–250.

Schek RM, Taboas JM, Segvich SJ, Hollister SJ, Krebsbach PH (2004). Engineered osteochondral grafts using biphasic composite solid free-form fabricated scaffolds. *Tissue Eng* **10:** 1376–1385.

Schek RM, Taboas JM, Hollister SJ, Krebsbach PH (2005). Tissue engineering osteochondral implants for temporomandibular joint repair. *Orthod Craniofac Res* **8:** 313–319.

Scheller EL, Krebsbach PH (2009). Gene therapy: design and prospects for craniofacial regeneration. *J Dent Res* **88:** 585–596.

Scheller EL, Krebsbach PH, Kohn DH (2009). Tissue engineering: state of the art in oral rehabilitation. *J Oral Rehabil* **36:** 368–389.

Seo BM, Miura M, Gronthos S *et al* (2004). Investigation of multipotent postnatal stem cells from human periodontal ligament. *Lancet* **364:** 149–155.

Shayesteh YS, Khojasteh A, Soleimani M, Alikhasi M, Khoshzaban A, Ahmadbeigi N (2008). Sinus augmentation using human mesenchymal stem cells loaded into a beta-tricalcium phosphate/hydroxyapatite scaffold. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **106:** 203–209.

Shen HC, Peng H, Usas A *et al* (2004a). Ex vivo gene therapy-induced endochondral bone formation: comparison of muscle-derived stem cells and different subpopulations of primary muscle-derived cells. *Bone* **34:** 982–992.

Shen HC, Peng H, Usas A, Gearhart B, Fu FH, Huard J (2004b). Structural and functional healing of critical-size segmental bone defects by transduced muscle-derived cells expressing BMP4. *J Gene Med* **6:** 984–991.

Shi S, Gronthos S, Chen S *et al* (2002). Bone formation by human postnatal bone marrow stromal stem cells is enhanced by telomerase expression. *Nat Biotechnol* **20:** 587–591.

Shi YY, Nacamuli RP, Salim A, Longaker MT (2005). The osteogenic potential of adipose-derived mesenchymal cells is maintained with aging. *Plast Reconstr Surg* **116:** 1686–1696.

Solchaga LA, Gao J, Dennis JE *et al* (2002). Treatment of osteochondral defects with autologous bone marrow in a hyaluronan-based delivery vehicle. *Tissue Eng* **8:** 333–347.

Sotille V, Thomson A, McWhir J (2003). In vitro osteogenic differentiation of human ES cells. *Cloning Stem Cells* **5:** 149–155.

Steiner C, Elixhauser A, Schnaier J (2002). The healthcare cost and utilization project: an overview. *Eff Clin Pract* **5:** 143–151.

Thomson J, Itskovitz-Eldor J, Shapiro S *et al* (1998). Embryonic stem cell lines derived from human blastocysts. *Science* **282:** 1145–1147.

Tong W, Brown SE, Krebsbach PK (2007). Human embryonic stem cells undergo osteogenic differentiation in human bone marrow stromal cell microenvironments. *J Stem Cells* **2:** 139–147.

Tu Q, Valverde P, Li S, Zhang J, Yang P, Chen J (2007). Osterix overexpression in mesenchymal stem cells stimulates healing of critical-sized defects in murine calvarial bone. *Tissue Eng* **13:** 2431–2440.

Ueno A, Kitase Y, Moriyama K, Inoue H (2001). MC3T3-E1-conditioned medium-induced mineralization by clonal rat dental pulp cells. *Matrix Biol* **20:** 347–355.

Ulrich-Vinther M (2007). Gene therapy methods in bone and joint disorders. Evaluation of the adeno-associated virus vector in experimental models of articular cartilage disorders, periprosthetic osteolysis and bone healing. *Acta Orthop Suppl* **78:** 1–64.

Voutetakis A, Zheng C, Metzger M *et al* (2008). Sorting of transgenic secretory proteins in rhesus macaque parotid glands after adenovirus-mediated gene transfer. *Hum Gene Ther* **19:** 1401–1405.

Wang X, Hu X, Daley A, Rabotyagova O, Cebe P, Kaplan DL (2007). Nanolayer biomaterial coatings of silk fibroin for controlled release. *J Control Release* **121:** 190–199.

Yoon E, Dhar S, Chun DE, Gharibjanian NA, Evans GR (2007). In vivo osteogenic potential of human adipose-derived stem cells/poly lactide-co-glycolic acid constructs for bone regeneration in a rat critical-sized calvarial defect model. *Tissue Eng* **13:** 619–627.

Zuk PA, Zhu M, Mizuno H *et al* (2001). Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng* **7:** 211–228.