Bone induction in clinical orthodontics: A review

Bone induction is needed to augment fracture healing and to fill osseous defects. Over the years autogenous cancellous bone grafting has been considered the "gold standard" in this form of treatment. Autogenous grafting has limitations, however, due to inadequacy of supply and surgical morbidity, including donor site pain, paresthesia, and infection, which can approach 8% to 10%. Moreover, graft resorption poses a severe problem. In an experimental study, endochondral bone grafts lost 65% of their original volume. Allografts, an alternative to autogenous grafting, seem to be biologically inferior and are associated with infection and inflammation.

To overcome this problem, investigators have attempted to develop synthetic composite grafts that are intended to mimic the natural components required for bone healing. Three general approaches have been developed: matrix-based therapies, factor-based therapies, and cell-based therapies.

Matrix-based therapies simply introduce biocompatible implants to replace the missing bone, and they consequently depend on the recruitment of endogenous osteoprogenitors to repair the osseous tissue. Some are based on titanium fiber metals and ceramics composed of hydroxyapatite, tricalcium phosphate, or both. Optimal, they have a porous nature that facilitates bony ingrowth and allows osteoconduction. They are not bone inductive but can be used as carriers for other bone-inductive techniques. Factor-based therapies provide direct osteoinductive stimuli. Growth and differentiation molecules, including demineralized bone matrix (DBM) or bone morphogenetic proteins (BMPs), have been introduced to bone defects. In the third approach—the cell-based therapies—the cells with osteogenic potential are transferred directly to the site requiring augmentation. They do not depend on local osteoprogenitors for the synthesis of new bone at the site of the defect and are particularly attractive for patients in whom the host tissue bed has been compromised.

The second and third approaches, which involve bone induction, will be the focus of this review.

Factor-based approaches

Numerous mediators have been implicated as the predominant growth factors in bone healing, with members of the transforming growth factor-β (TGF-β) superfamily of polypeptide growth factors being the most notable. Its members include TGF-β1 through TGF-β3 and other peptides, including the BMPs and growth differentiation factors (GDFs). This group of growth factors appears to regulate the...
proliferation and expression of differentiated phenotypes for many cell populations, including chondrocyte, osteoblast, and osteoclast precursors. The extracellular matrix of bone is the largest source of TGF-βs in the body. TGF-βs are also present in the graft hematoma after release by the platelets and are also synthesized by mesenchymal cells.

Other growth factors present in the cal- lus during the bone healing process include fibroblast growth factors (FGFs), platelet-derived growth factors (PDGFs), and insulin-like growth factors (IGFs). FGFs are well-known mitogenic and angiogenic factors that are important in neovascularization and wound healing. PDGFs act as local tissue growth regulators and initially were isolated in blood platelets, underscoring one of the important roles of the clot in fracture healing. IGFs are another example of matrix-synthesizing growth factors that are important in bone healing.

**Bone morphogenetic proteins**

Among the growth factors tested in heterotopic and orthotopic locations, BMPs, either in native or recombinant forms (rhBMPs), appear to be the most promising. Urist made the key discovery that demineralized bone segments and extracts of demineralized bone induce bone formation when implanted subcutaneously or intramuscularly in animals. Subsequent purification studies of these bone-inductive proteins resulted in the identification of many members of BMPs.

BMPs are low-molecular-weight glycoproteins and have a pleomorphic function that ranges from extracellular and skeletal organogenesis to bone generation and regeneration. Bone induced by BMP in postfetal life recapitulates the process of embryonic and endochondral ossification. BMPs are important regulators in osteogenic differentiation during fracture repair. Wang and coworkers showed that BMP-2 caused commitment and differentiation of multipotent stem cell line into osteoblast-like cells. Clinically, native human BMP has been used successfully for the treatment of established nonunions and spinal fusions.

BMP-4, BMP-6, and to a lesser extent BMP-5, have also been shown to induce new bone formation.

Through recombinant gene technology, BMPs are available in sufficient amounts for basic research and clinical trials. RhBMP-2 and rhBMP-7 induce structurally sound orthotopic bone in various experimental systems. These BMPs have the capability of healing critical-size defects in rodents, dogs, sheep, and primate models when combined with collagen, DBM, or biodegradable polymers.

Zegzula and coworkers successfully induced bone formation with restoration of cortices and marrow elements with the use of rhBMP-2 delivered in porous poly(DL-lactic acid) implants in rabbits. Studies reported to date with rhBMPs have been related largely to animals, although clinical trials currently are underway in the United States and in Europe.

**Demineralized bone matrix**

DBM is an osteoinductive material that is developed to a stage that can be used routinely in clinical situations. It is considered an acceptable alternative to autogenous grafting in various craniofacial, oral surgical, periodontal, and hand-reconstructive procedures. Implantation of DBM in segmental defects in rats resulted in complete regeneration in less than 8 weeks. At 12 weeks after the operation, the mechanical properties of the new tissues were comparable to bone formed in the early stages of normal fracture repair. The capacity of the rat to regenerate bone in response to DBM is extraordinary; such implants induced repair of segmental fibular defects that were too large to be healed even by autogenic bone grafts. Clinically, the use of DBM in patients in the form of pulverized bone has been shown to predictably induce osteogenesis and thus bone healing in craniofacial defects. The bone-induction capacity of DBM has been attributed to its content and the diffusibility of BMPs and other cytokines that interact with the undifferentiated osteogenic precursor cells in the host bed and cause them to differentiate into functionally active osteogenic elements. The BMP was found to diffuse...
to significant distances along surfaces of contact cells with bone matrix. These distances, measured by experiments on implants in diffuse chambers, were as great as 1000 µm. Rabie and Urist examined the vascular endothelial growth pattern during DBM-induced osteogenesis. The results demonstrated rapid vascularization, which is vital to the healing and bone-induction ability of DBM. Rabie and coworkers recommended the use of intramembranous bone with DBM in clinical cases where earlier loading is needed. Ultrastructural identification of cells involved in the healing of intramembranous and endochondral bone showed that the former healed through an osteogenic ossification route, without the presence of a cartilage stage.

DBM has limitations. Reconstruction of large skeletal defects by implantation of DBM without a skeletal substitute has been associated with incomplete bridging in 19% and 25% of the implants, respectively, using either allogenic or autogenic DBM. Composite bone grafts, to be discussed later, were developed to overcome this problem.

**Carriers for growth factors**

Numerous biodegradable and ceramic carriers have been used with varying degrees of success as delivery vehicles or carriers for various growth factors, most notably the BMPs. Some investigators have found that polylactic and polyglycolic acid porous microspheres, when combined with an appropriate dose of rhBMP-2, appear to be as effective as inactivated DBM with BMPs. Zegzula and coworkers successfully induced bone formation with restoration of cortices and marrow elements with the use of rhBMP-2 delivered in porous poly(DL-lactic acid) implants in rabbits. Others have used collagen matrices as successful carriers for BMPs. The activity of BMPs is affected critically by the carrier. Ceramic carriers have been used, but their extremely slow biodegradability makes them suboptimal as carriers. Radomsky and coworkers reported the effectiveness of an injectable bFGF combined with sodium hyaluronate gel in stimulating fracture healing.

Overall, the development of appropriate osteoconductive carriers has not progressed as rapidly as the isolation and synthesis of the growth factors. Unfortunately, this has significantly slowed the development of clinically successful biosynthetic bone grafts.

**Cell-based approaches**

Thus far, 4 different cell-based tissue-engineering approaches have been described. They involve the implantation of: (1) unfractionated fresh bone marrow; (2) purified, culture-expanded mesenchymal stem cells; (3) differentiated osteoblasts and chondrocytes; and (4) cells that have been modified genetically to express a rhBMP.

**Unfractionated fresh bone marrow**

The first cell-based approaches for tissue engineering of bone used unfractionated fresh autologous or syngeneic bone marrow. Because bone marrow is known to contain osteogenic precursors, its implantation was perceived to have the potential to lead to effective bone regeneration. Various preclinical investigations and a limited number of clinical studies have confirmed this to be true. In a clinical setting, autologous marrow is harvested from the iliac crest and immediately transplanted to the site in need of skeletal repair. Fresh marrow also has been used as a cellular source to grow bone at an ectopic site before orthotopic implantation.

Despite the successes that have been obtained using fresh marrow transfer, a single biologic consideration limits its widespread application. That is, it is frequently impractical to obtain enough bone marrow with the requisite number of osteoprogenitor cells. The reduction in healthy marrow elements that occurs as a consequence of aging or disease is accompanied by a diminution of the cellular constituents, especially the osteogenic precursors. In the best-case scenario, osteoprogenitors represent approximately 0.001% of the nucleated cells in healthy adult marrow.
Mesenchymal stem cells

Isolated adult human and animal mesenchymal stem cells from bone marrow and periosteum have the capacity for extensive replication without differentiation, and they process a multilineage developmental potential, allowing them to give rise to not only bone, but also cartilage, tendon, muscle, fat, and marrow stoma. Techniques have been developed to allow these cells to be cultured and multiplied extensively without undergoing differentiation. With these culture systems, these cells from a small marrow aspirate of an adult can be passaged for more than 30 population doublings in vitro and expanded in number more than 1 billionfold. The phenotype of the cells is stable throughout culture and there is no loss in osteogenic, chondrogenic or adipogenic potential. This dramatic expansion makes mesenchymal stem cells a clinically useful source of progenitor cells for tissue engineering of bone and other mesenchymal derivatives.

A series of preclinical studies using mesenchymal stem cells in a porous hydroxyapatite/tricalcium phosphate carrier to achieve osseous regeneration in a segmental critical sized defect of the femur was performed. With rat, canine, or human mesenchymal cells, bone fill ranged from 40% to 47% of available space, a value not likely to be exceeded given the nature of the scaffold and the requirement for vasculature and associated soft tissue.

Differentiated osteoblasts and chondrocytes

When mesenchymal stem cells are used for the treatment of bone defects, differentiation of the stem cells must occur after implantation of the construct. In an attempt to accelerate and enhance bone formation, several investigators have explored the use of differentiated, or secretory, osteoblasts in tissue engineering of bone. Culturing mesenchymal stem cells with dexamethasone, ascorbic acid, and β-glycerophosphate directs the cells into the osteogenic lineage. Theoretically, these predifferentiated osteoblasts can then be used to treat various bone defects.

Okumura and coworkers and Yoshi-kawa and coworkers cultured bone marrow–derived rat mesenchymal stem cells with dexamethasone on porous coralline hydroxyapatite to permit attachment of the cells to the matrix and promote lineage progression, while still in vitro. Implantation of the differentiated cell and matrix combination into an ectopic subcutaneous site supported an accelerated bone formation compared with samples implanted with undifferentiated marrow cells. Breitbart and coworkers also used undifferentiated cells to achieve tissue-engineered bone repair. For these studies, a nonwoven, resorbable polyglycolic acid fiber was used as a scaffold for cell delivery. The fibers were seeded with cells derived from rabbit periosteal tissue, and were cultured with dexamethasone in vitro to promote osteoblastic differentiation. The osteoblast-seeded fibers were used to treat full-thickness calvarial defects in rabbits. Twelve weeks after implantation, the cell-seeded fibers produced significant bone, whereas little repair was seen in defects that had been left untreated or were implanted with fibers not containing cells. Together, these studies suggest that committed osteoblasts could form the basis for rapid and effective repair of bone defects.

Because autologous osteoblastic cells are considerably more difficult to obtain than mesenchymal stem cells, and because even after isolation they possess a limited capacity for proliferation, the only tenable solution is to generate mature osteoblastic implants from culture-expanded progenitor cells.5

Because endochondral bone formation, and frequently fracture repair, proceeds through a cartilaginous intermediate stage, some have thought it possible that the transplantation of committed chondrocytes also would lead to bone regeneration. To test this hypothesis, Vacanti and coworkers compared the ability of periosteal progenitors and articular chondrocytes to effect bone repair. Both formed cartilage, but no endochondral ossification occurred in the latter samples. Therefore, chondrocytes proved ineffective as a cell-based therapy for tissue engineering of bone.
Genetically modified cells (gene therapy)

Gene therapy involves the manipulation of endogenous cells to generate specific proteins. By transferring genes into cells at a specific anatomic site, the osteoinductive properties of growth factors can be used at physiologic doses for a sustained period to facilitate a more significant healing response. To achieve gene transduction of a target cell, gene therapy models use vectors to enhance the entry and expression of exogenous DNA into the target cells nucleus. Gene therapy vectors delivered to a treatment site in osteoconductive carriers have yielded promising results in animal models. They used cells simply as a delivery vehicle for the genetic material coding for a BMP. An immortalized stromal cell line was infected in vitro with an adenovirus construct containing rhBMP-2 cDNA. The cell line was shown to transiently secrete rhBMP-2, with minimal production only 7 days after infection. This transient expression, although problematic for many systemic gene therapy applications, may be appropriate for the treatment of bone defects, where short-term expression of the protein should be sufficient. The BMP-2–producing cells were mixed with a scaffold of devitalized DBM and used to repair a segmental bone defect in young athymic rats. Significant new bone formation was seen in animals that had been treated with the genetically modified cells. Transfer of the BMP-2–producing cells induced the formation of coarse trabecular bone, whereas lacelike lamellar bone resulted from the direct implantation of rhBMP-2 protein. These results suggest that cells may serve as effective vehicles for providing regional gene therapy in the repair of osseous defects.

Combination approaches

Composite biosynthetic bone graft

Lane studied whether the osteoprogenitor cells and the osteoinductive rhBMP-2 were synergistic when used in combination. Critical-size defects were tested with bone marrow alone, rhBMP-2 alone, and both substances together, all in the presence of an established inert biodegradable matrix. The result showed the clear superiority of the rhBMP-2 combined with bone marrow and polylactic-glycolic acid carrier over all the other groups in terms of bone formation, union, and biomechanical strength. Although in the absence of bone marrow, BMPs can lead to successful healing through the stimulation of local mesenchymal stem cells, the coinsertion of osteoprogenitor cells that respond to the osteoinductive growth factors significantly enhances the bone repair process. Now undergoing clinical trials in humans, composite synthetic bone grafts using osteoinduction, osteoprogenitor cells, and biodegradable osteoconduction have shown synergism between components and were superior to the “gold standard” autogenous bone graft.

Composite bone graft

The composite bone graft was presented by Rabie and coworkers in 1996 as autogenous bone graft (intramembranous or endochondral in origin) mixed with DBM. This composite bone graft produced 47% more bone than autogenous endochondral bone alone. One possible hypothesis of the composite bone graft, as outlined by Rabie and Lie, was that in the composite bone graft, the bone-induction capacity of bone grafts was optimized by surrounding them with osteoinductive DBM. On the one hand, the bone graft was considered a scaffolding, potential carrier, and defect-filling material that prevented tissue collapse as occurred on DBM grafts alone. On the other hand, the advantage of DBM was ascribed to its content and the diffusibility of the BMPs and other cytokines, which interacted with the undifferentiated osteogenic precursor cells in the host bed and caused them to differentiate into functionally active osteogenic elements. This composite bone graft has already been used successfully clinically and will be discussed later in the clinical application section.
Altering of physical environment

Tension across fractured site (distraction osteogenesis)

Distraction osteogenesis is a well-established technique for bone lengthening that has widespread clinical applications in the treatment of limb length discrepancies, bone defects, limb deformities, and fracture nonunion. The principles of this method were developed by Ilizarov in the early 1950s. An osteotomy is performed, followed by fixation with an external fixator. After a latency period of about a week, the osteotomy is subjected to controlled distraction. Osteogenesis is thereby induced and the bone continues to grow as long as the distraction is maintained at an adequate rate. When distraction is stopped, bone lengthening ceases and the newly formed bone in the distracted zone gradually consolidates. Ilizarov called the biologic consequences of these mechanical changes the "tension-stress effect." A fibrous interzone develops in the gap between the bone fragments, and this fibrous tissue is transformed into bone tissue. This occurs either directly by intramembranous ossification or indirectly through a cartilaginous intermediary. Rauch and coworkers demonstrated that there was a high level of expression of BMP-2, BMP-4, and BMP-7 during the entire distraction phase; that expression gradually disappeared during the consolidation phase in rabbits. These results are compatible with the hypothesis that BMPs play an important role in the signaling pathways that link the mechanical forces created by distraction to biologic responses.

Tension across suture

Mechanical stress is known to influence both formation and bone resorption. Ikegame and coworkers used an organ culture system in which mouse calvarial sutures were incubated with or without tensile stress. They showed that tensile stress application to mouse calvarial sutures induced differentiation and growth of osteoblasts, which eventually led to osteogenesis. Also, BMP-4 may play a role as an autocrine and paracrine factor in the differentiation of preosteoblasts and fibroblasts into osteoblasts, thereby stimulating osteogenesis induced by tensile stress in the organ culture of the calvarial suture.

Clinical experiences of bone induction in relation to orthodontic treatments

Repair of alveolar bony defects

Alveolar bony defects can be created after extractions for orthodontic treatment. Repair of the defects is needed to avoid the risk of dehiscence and other periodontal insults at a later stage after the teeth have been retracted into the extraction site. Rabie and Chay reported the use of composite bone grafts for the repair of alveolar bony defects. An accidental loss of the buccal plate of bone occurred during extraction of a buccally placed premolar for orthodontic purposes. The defect was repaired using chin bone mixed with DBM, and the teeth were successfully moved into the grafted area. A 5-year follow-up showed a stable gingival condition at the grafted area.

Alveolar ridge augmentation for implants and orthodontics

Collapse of the alveolar ridge, eg, after trauma, poses a problem for implant placement for restoration of function and esthetics. Ridge augmentation would be needed for implant placement and orthodontic treatment. Rabie and Urist illustrated a case of sport injury causing loss of 3 mandibular incisors. The extent of the associated buccal bone loss prevented conventional implant treatment. Ridge augmentation with the composite DBM and intramembranous bone was carried out for implant placement and orthodontic treatment. Orthodontic treatment was required to redistribute the space for the dental implants. A 3-year follow-up showed stability of the results.

Bone induction and orthodontics in the treatment of periodontal disease

Cortellini and Bowers examined clinical and experimental outcomes for the
treatment of intrabony defects and searched the literature for techniques that would most predictably achieve desired treatment goals. It was concluded that guided tissue regeneration (GTR), GTR combined with the use of DBM, and DBM alone are the most predictable regenerative procedures for achieving selected treatment outcomes. They pointed out that defect selection is critical to achieving a successful outcome; deep and narrow defects showed the most predictable positive response to regenerative procedures. Rabie and coworkers showed that orthodontic intrusion can change a horizontal bony defect into a deep and narrow defect that is more favorable for regeneration of the periodontium through grafting procedures. A typical treatment plan of such a case included periodontal treatment, including scaling and root planing before orthodontic intrusion; adjunctive orthodontic intrusion with a simple fixed appliance to intrude and align the maxillary left central incisor and to create a vertical defect; periodontal surgery, packing DBM around the intruded tooth to regenerate bone into the vertical defect; and finally, retention with a fixed lingual retainer.

Repair of alveolar clefts

Patients with cleft lip and palate often require a secondary bone graft procedure to allow eruption of anterior teeth and orthodontic treatment. Severe bone resorption sometimes occurs after bone grafting. Composite bone graft can be used to improve the healing. Rabie and Chay reported of a case of bone resorption after bone grafting of a bilateral cleft lip and palate patient. A second surgery to augment the ridge was carried out; this time, bone harvested from the chin mixed with DBM was used. The 3-year postoperative result showed that the use of intramembranous chin bone mixed with DBM demonstrated faster healing and better integration of the bone graft with the host bone.

Jaw reconstruction

A common application of the composite autogenous bone graft containing cortico-cancellous bone mixed with DBM is in the area of maxillary and mandibular reconstruction. After tumor resection, skeletal support in the form of free bone block may be used in conjunction with particulate bone mixed with DBM. The newly formed bone unites the autogenous bone, the DBM, and the recipient bed. This amalga-mation is indicative of an active state of remodeling and incorporation that allows earlier loading. Earlier loading helps to decrease bone resorption at the grafted site. Clinical experience shows that endosseous dental implants can be placed 3 to 4 months after the grafting procedure.

Conclusions

Bone induction using DBM has a wide range of clinical applications in orthodontics. Other techniques of bone induction are now being developed, and we hope new materials for clinical use can appear in the market in the near future.

References


