Osteobiologics

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Abstract

“Osteobiologics” is the term that has been introduced to refer to the class of engineered materials that have been created and which promote healing of fractures and bone defects. The list of osteobiologics is rapidly expanding as new products incorporating osteoconductive materials are mixed with a variety of osteoinductive proteins, demineralized bone, and preparations of osteogenic cells. The growth in osteobiologics has been stimulated by the early success of osteoconductive materials as graft substitutes in the repair of fractures and by the increasing demand for grafts in all areas of orthopaedics. Although allografts have historically been employed with success, the number of donors has grown much slower than demand leading to the development of artificial materials. Manufactured bone graft substitutes, or osteobiologics, attempt to mimic the components of an autogenous bone graft by reproducing the bone matrix, which is osteoconductive and osteoinductive. Other products aim to introduce osteogenic cells by concentrating bone marrow while others introduce differing growth factors from platelets in peripheral blood. Very few of these products have been supported by appropriate clinical studies and as such their value is unknown. Orthopaedic surgeons employing these products must understand the basic science principles behind their development in order to understand the indications and limitations of their application. Properly designed clinical studies should be performed to determine the usefulness and cost-effectiveness of both current and future products.

The goals of present day fracture management aim to secure complete healing of the fractured bone as well as complete return of function to the injured extremity. To achieve this, the surgeon will usually resort to operative fracture repair with closed or open reduction and internal fixation. In cases where there is significant comminution or crushing leading to loss of bone substance or with known problem fractures, bone grafting will often be used to replace lost structure and to improve the likelihood of rapid healing. Fracture healing requires stability as well as a structure to support the in-growth of the fracture-healing callus. In addition, growth factors and other active molecules within the fracture site released as a consequence of the injury act as signals to coordinate the process of fracture repair. Above all, mesenchymal cells must be present which act to produce the callus and remodel the matrix throughout the process of repair until complete bone healing is achieved. Bone graft material assists this process by providing a three dimensional structure as well as a matrix that contains growth factors and other materials that cause uncommitted primitive cells to develop the osteoblastic phenotype. Fresh autograft also provides living osteoblastic cells that can promote the process of healing. Since autogenous graft provides these three ingredients, it is described as being osteoconductive, osteoinductive, and osteogenic. Autogeneous cancellous bone harvested from the iliac crest provides these three factors. To date no bone graft substitutes can match this. As a result, autogenous cancellous bone is still considered the “gold standard” bone graft material.

Unfortunately, there is considerable morbidity associated with autograft harvest. Complications such as infection, wound hematoma and drainage, severe pain, and even iliac wing fracture occur with regularity. In many cases the donor site may not provide sufficient material for the needs of the reconstruction. This is especially true in the elderly and in children. In an effort to avoid these complications, ortho-
paedic surgeons explored the use of substitutes ranging from calcium sulfate to boiled xenographic bone. Allograft bone has been used extensively and has been especially useful as a bulk, structural replacement in cases of segmental skeletal loss.

The demand for bone grafts has increased significantly in recent years largely due to the growth of spinal fusion procedures. It is estimated that approximately 500,000 grafting procedures will be performed each year of the coming decade in the United States alone. This demand vastly outstrips the supply of allograft donors so that allograft materials cannot be expected to meet demand4 (Fig. 1). This has spurred the development of synthetic graft substitutes with the expectation that they will function as well as autograft while being provided with adequate supply at reasonable cost. The bone graft substitutes that are currently available can be classified as osteoconductive matrix materials, demineralized bone matrix products, and osteoinductive proteins and carrier produced from recombinant DNA processes.

**Osteoconductive Matrix Materials**

The term “osteocduction” applies to the three dimensional process observed when porous structures are implanted in or adjacent to bone.6 Capillaries, perivascular tissues and osteoprogenitor cells migrate into the porous structures and incorporate the porous structures with new-formed bone. Osteoconductive substrates are not viable, but are passive scaffolds into which grows the osteoprogenitor tissue. It is clear that the chemical nature of the osteoconductive material is an important influence on the degree to which tissue grows into the porous matrix as well as the amount of new bone that is formed within it. Porous structures, which have structures similar to cancellous bone, are more completely incorporated than less porous structures. Osteoconductive matrices with chemical structures similar to bone matrix, in addition to having beneficial porosity, also mimic exposed matrix activating osteoclastic remodeling. This remodeling process results in turnover of the material with significant new, host bone formation. The ideal osteoconductive matrices are therefore porous structures of calcium phosphate with or without type I collagen.3,6

Currently, there are a variety of calcium phosphate products that have been produced to act as bone graft substitutes. These products vary from structural, insoluble hydroxyapatites to highly soluble non-structural calcium sulfates. The different products have significantly different properties, degrees of incorporation, and rates of turnover into host bone.

Highly crystalline hydroxyapatites were the first products introduced to the market. These were prepared from the skeletons of South Sea corals that were chemically converted to pure hydroxyapatite. These products, examples of which are Pro-Osteon and Interpore, have compressive strength similar to cancellous bone and have been used successfully to replace structural loss in metaphysical regions. A very useful application for these materials has been as graft substitutes used for reconstruction of the metaphyseal defects associated with tibial plateau fractures.7 These highly crystalline materials, however, are essentially insoluble and are not resorbed by osteoclasts. These are therefore permanent implants, which are not re-modeled into host bone. As a result, there has been reluctance to use these materials in cortical sites where complete remodeling of a defect or fracture into native lamellar bone is critical to the biomechanical integrity of the site. Use of a hydroxyapatite material will prevent complete remodeling leaving the grafted region subject to the possibility of a repeat fracture.

Other products are produced from calcium sulfates or tri-calcium phosphates. Calcium sulfate is highly soluble. Implants prepared from calcium sulfate rapidly disappear.
with ingrowth of new-formed bone at the implantation site.\textsuperscript{9} Tri-calcium phosphate can be formed into highly porous implants, which have some compressive strength (Fig. 2). When tri-calcium phosphate implants are implanted into orthotopic sites they are rapidly invaded by a fibrovascular stroma that is soon followed by the appearance of osteoblastic and osteoclastic cells that begin to lay down new bone on the implant as it is rapidly remodeled\textsuperscript{9} (Fig. 3). This process appears histologically similar to "creeping substitution." Tri-calcium phosphates are incorporated quickly but are remodeled more slowly than calcium sulfates allowing them to fulfill a support function for a longer period of time. Surgeons are now able to choose a substitute graft material with this in mind. If a clinical scenario calls for grafting a site that does not require physical support, then calcium sulfate products may be the most desirable for their rapid dissolution and replacement by bone. If, however, physical support is required, then the surgeon should choose a more slowly remodeled material of calcium phosphate, which will provide support for longer as the graft is slowly remodeled into host bone. Hydroxyapatite products, which do not remodel, provide for the longest period of support, but are probably no longer indicated in view of the tri-calcium phosphate products that are now available.

The introduction of calcium sulfate and calcium phosphate cements is a recent advance in this field.\textsuperscript{10-12} When prepared, these cements are initially liquid and can be injected into fracture sites where they cure and become hardened (Fig. 4). Their advantage is that very complete filling of defects can be achieved. In fact, if all loose cancellous bone is first removed before filling the defect with one of these cements, the support function of the cement reconstruction is superior to that which can be achieved with cancellous graft.\textsuperscript{12} These cements additionally, cure isothermically, which is an advantage compared to using methylmethacrylate. There is some experience that these cements can even improve the purchase power of stripped screws. It is hoped that with continued development, these cements will replace methylmethacrylate completely for augmentation of fracture sites and osteoporotic bone.

Type I collagen, usually produced from bovine sources, has also been developed for use as a bone graft substitute. Collagen is osteoconductive due to its three-dimensional structure, but it also binds circulating growth factors and other molecules that enhance new bone formation. When added to calcium phosphate ceramics the resulting composites usually enhance the graft function,\textsuperscript{13} especially when mixed with bone marrow or autogenous, cancellous bone. It may be that collagen can confer some osteoinductive properties as a result of binding circulating growth factors and other molecules important to new bone formation. Collagen-calcium phosphate composites have been in use for over a decade and have demonstrated clinical usefulness equivalent to autogenous graft in reconstruction of fractures and bone defects.\textsuperscript{14}

**Osteoinductive Bone Graft Substitutes**

"Osteoinduction" is the term used to describe the process by which primitive, pluripotential, mesenchymal cells are induced by exposure to various growth factors to mature into osteoblastic phenotypes.\textsuperscript{15} The purest expression of these phenomena is heterotopic bone formation. In fact, current bioassays, which test for osteoinduction in graft materials, use rodent muscle pouch models, which quantify the degree of bone formation within the skeletal muscle formed in response to the inductive implant.\textsuperscript{16} Marshall Urist is credited with this discovery as he recognized it as a characteristic of demineralized bone. Bone, which is demineralized in 0.5 N
HCl, results in demineralized bone matrix (DBM). DBM is composed of approximately 90% type I collagen and 10% non-collagenous proteins, including several growth factors making up the bone morphogenetic proteins. DBM implants are osteoconductive and are osteoinductive in most animals and possibly in humans. Freshly prepared and carefully preserved DBM successfully heals critical defects in most animal models. The osteoinductive capacity of DBM can be affected by preparation and storage and appears to vary between human donors. There is ample evidence that many of the DBM products that are currently available are excellent graft substitutes especially when implanted into contained bone defects, fractures, or arthrodeses combined with stable internal fixation. Unfortunately, there are no well-designed studies that document the efficacy of DBM in the treatment of non-unions, a clinical scenario where osteoinduction would be a theoretical advantage. In my own experience, there appears to be a significant variability of osteoinductive capacity between donors. The best application of DBM may be when it is combined with autogenous bone or marrow acting to expand the volume of the graft.

Bone morphogenetic proteins (BMP) can be manufactured by recombinant DNA technology. Currently, two BMPs, BMP2 and BMP7, are prepared for clinical use combined with collagen carriers. There is at present some compelling preliminary evidence that BMP2 and 7 may enhance fracture healing in non-unions and complex open tibial fractures, however definitive studies are still pending.

**Osteoprogenitor Cells**

Bone marrow has been used to enhance fracture healing for nearly two decades. Studies by Connolly and Werntz document the potential for bone marrow cells to improve healing of fractures and nonunions as well as critical defects in animal models. Bone marrow aspiration is an inefficient method of obtaining osteoprogenitor cells because most aspirates are largely red blood cells. There are devices available that are intended to concentrate blood or marrow increasing the yield of osteoprogenitor cells but their efficacy is untested. This is clearly the missing link in bone graft substitute development. The most challenging fractures and nonunions are those where blood supply or healing potential are deficient. What is lacking in most chronic nonunions are viable cells upon which osteoinductive factors can act. Clearly, a site deficient in pluripotential cells that has been replaced by mature fibrous scar will not respond to graft substitutes unless osteoprogenitor cells are introduced. The next advance in this field awaits development of implants containing mesenchymal stem cells or even cells genetically manipulated to produce supra-physiologic amounts of bone morphogenetic proteins.

**Summary**

The most important factors that must be restored in the treatment of fractures, delayed unions, and nonunions are proper alignment and mechanical stability with the preservation of the blood supply. Failure of fracture healing usually results from inadequate mechanical stability at the fracture site, poor biology, or both. Bone grafting of fractures or nonunions without first achieving proper stability and blood supply for that fracture will be unsuccessful. Once stability and blood supply adequate to promote healing are achieved, the surgeon can make the decision whether or not a bone graft or graft substitute will be helpful.
The decision to use a bone graft or graft substitute must be based upon the clinical problem: is there a loss of structure or a lack of osteoinduction or osteogenesis. Autogenous bone graft is still the gold standard, and the decision to use a substitute is usually based on the fear over the lack of donor site graft or an attempt to avoid donor site morbidity. Well-contained defects and acute fractures can usually be treated with an osteoconductive implant, usually from the family of calcium phosphate or calcium sulfate products. Well-contained defects provide a vascularized, stable environment and acute fractures generally are rich in cells and osteoinductive signals. In these settings osteoconductive products work well. For intra-articular fractures with joint surface depression and crushing of metaphyseal bone, both calcium phosphate and calcium sulfate cements have demonstrated clinical effectiveness that may surpass cancellous autograft. Fractures that are at risk for poor healing such as open fractures with soft tissue loss, atrophic nonunions, and those with significant loss of substance need more than osteoconduction alone. This is the setting for agents that provide cells as well as osteoinductive signals that can regenerate the osteogenic substrate at the fracture site. At the present time there is no graft substitute that can fully provide this requirement. The clinical studies performed to date may suggest that BMP2 or BMP7 may be helpful, but definitive data is lacking. Until such evidence is available, surgeons in practice should rely on autogenous grafts that can be supplemented with an osteoconductive matrix or DBM or other inductive product (Fig. 5). To quote the recommendation of the AAOS Committee on Biological Implants: “A Quandary of Choice confronts the orthopedic surgeon. Caveat Emptor! Selection should be based on reasonable burdens of proof. These include examination of the product claims and whether or not they are supported by preclinical and human studies in site specific locations where they are utilized in surgery.”

References