A Review Paper

Osteobiologics

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Abstract

Osteobiologic adjuvants that aid in bone grafting have recently been popularized. Current osteobiologic technologies can be organized into 3 main categories: osteoconductive, osteogenic, and osteoinductive. Appropriate use of osteobiologic agents mimics autograft. Compared with autograft , synthetic adjuvants minimize donor morbidity. Understanding how synthetic agents can enhance bone formation and their appropriate use can aid the orthopedic surgeon in delivering optimal care in these difficult cases. The understanding of how synthetic grafts can enhance the normal bone healing cascade defines their role and use in treating fracture gaps.

Nationally, bone grafting accounts for more than 450,000 isolated grafting procedures a year. Bone grafting and various forms of fixation account for 2.2 million procedures a year. In total, the business of bone grafting represents approximately a $2.5 billion industry.[1] The goal of bone grafting in orthopedics is to generate an enchondral bone healing response, which by definition involves stem cells forming a cartilage precursor prior to ossification of the fracture gap. This paper presents an overview of the various osteobiologic agents that are currently used to supplement or replace autogenous bone graft.

During the stages of fracture healing (hematoma/inflammatory phase, reparative phase, soft callus phase, hard callus phase, and remodeling phase), various osteobiologic factors are naturally expressed. Both Cho[2] (Figure 1) and Morone[3] (Figure 2) have described various osteobiologic growth factors expressed during normal bone healing. During enchondral bone healing in the murine and human model, respectively, osteobiologic factors are expressed in a temporal as well as in a biphasic fashion. In Morone's studies using a spinal fusion model for bone morphogenic protein (BMP) gene expression in humans, he noted that not only are BMP 2, 4, and 6 expressed temporally, but the expression of these genes in a fusion model is biphasic (Figure 2).[3] Because these biologic factors are essentially proteins, it can be argued that many osteobiologic agents used as external adjuvants to grafting have inherent positive and negative feedback loops depending on the concentration and timing of their introduction during the healing cascade.

Given the fact that certain BMPs are expressed temporally and biphasically in normal healing, one must be aware that when these same agents are introduced into the graft site, they can either positively or negatively influence the formation of bone. One needs to express these factors artificially in either a positive feedback loop or a negative feedback loop.[2,3] According to Cho and colleagues, several members of the transforming growth factor-beta (TGF beta) family are involved in fracture healing, and, even though closely related both structurally and functionally, each possesses a distinct temporal expression pattern and a potentially unique role in fracture healing.[2]

The gold standard for bone grafting in orthopedic applications has been autograft.[4,5] Recent uses of osteobiologics in fracture healing have challenged this gold standard.[5-7]

The ideal graft consists of a triad of osteobiologic criteria (Figure 3). These characteristics would include osteogenic factors, osteoinductive factors, and osteoconductive factors. It is important to note that regardless of what kind of grafting material is used, the first step in grafting is to débride the fracture site.[5,8] Débriding the nonhealing fracture site is crucial—not only to remove fibrous interposition but also to generate a vascular response at the fracture ends.[9] The ideal graft needs a bleeding interface at the fracture ends in a time-dependent manner to serve as a "conduit" for fracture healing.[8] For any bone graft to successfully incorporate, there must preexist a vascular channel for osteoconductive, osteoinductive, and osteogenic adjuvants to work. This vascular highway is essential and is integral during the debridement process. The physical act of débridement to bleeding margins at the fracture or nonunion ends enables a preliminary vascular ingrowth to develop a vascular pathway at the fracture gap.

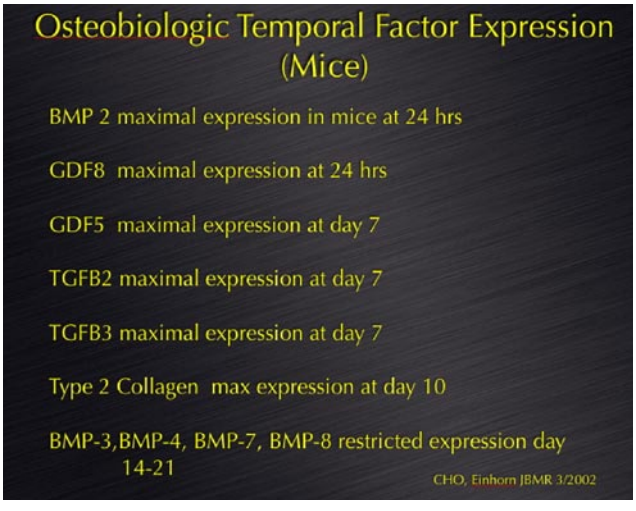

Figure 1. Temporal expression of osteobiologic growth factors in a murine model for fracture healing. From Cho et al.[2] Reprinted with permission from the American Society for Bone and Mineral Research.

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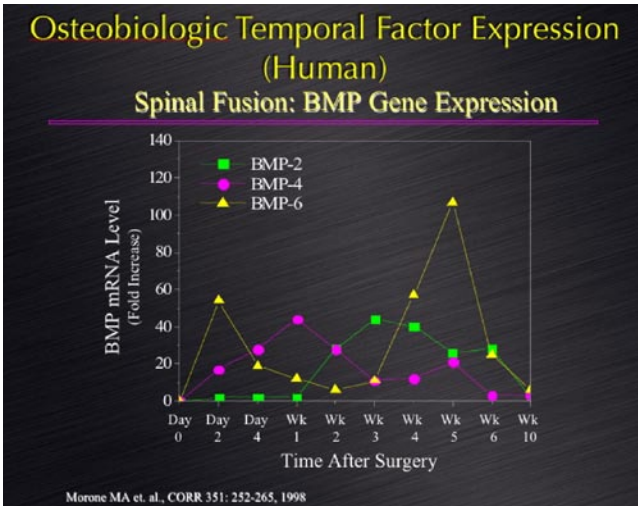

Figure 2. Temporal expression of osteobiologic growth factors in a human spinal fusion model. From Morone et al.[3] Reprinted with permission from Lippincott Williams & Wilkins.

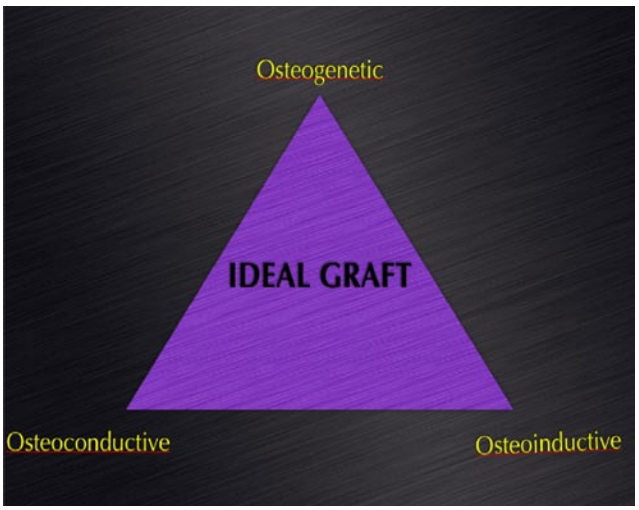

Figure 3. Triad of criteria for autograft and osteobiologic grafting.

Each of these characteristics of the ideal bone graft should be defined: *Osteogenicity* is the ability of the graft to have actual cells that can generate the healing cascade (osteoprogenitor cells). *Osteoinductivity* is the ability of the graft to induce undifferentiated stem cells and osteoprogenitor cells to generate a healing response. The ideal test of osteoinductivity is placement of an osteoinductive agent in a nonbone environment, ie, muscle.[10] According to Urist, the presence of that osteoinductive material in an ecotopic nonbone medium should generate bone. A material that is osteoinductive will generate a healing cascade even in a nonosseus bed. *Osteoconductivity* is the ability of the graft to have scaffolding that mimics subchondral cancellous bone porosity of approximately 150-600 microns. This cross-sectional pore size is essential for an optimum osteoconductive surface, as this porosity allows maximal vascular in growth.[11,12] This vascular ingrowth allows for the formation and nutritional support for the osteoprogenitor cells to initiate and help maintain the healing cascade within the graft bed.

Autograft

Although autograft is inherently osteogenic because it contains progenitor cells from the harvest site, the variability in the amount and quality of the graft can often lessen autograft's capacity to bridge a fracture gap. Typical autograft harvest sites are the anterior pelvis, posterior pelvis, proximal and distal femur, proximal and distal tibia, distal radius, and calcaneus. Inherent complications in general harvest morbidity are infection, prolonged wound drainage, hematoma formation, neurological and vascular damage, muscle herniation, subluxation of sacroiliac (SI) joints, destabilization of SI joint, and fractures from overzealous harvesting.[13,14] Osteoconductivity by definition is maintained during autogenous bone grafting because the primary harvest site is cancellous bone, with its optimal porosity. Osteoinductivity is maintained with autograft because the harvest is also taking progenitor cells from the marrow with its inductive agents. The issue with autograft is 2-fold: the quality of the harvest and the variability of the harvest concentration of progenitor cells. When one takes autograft either from bone marrow aspiration or from direct corticocancellous harvesting, the issue is uniformity of concentration.

Findings by Muschler and colleagues[6] clearly demonstrate that, during bone marrow aspiration, there is an 80% variability in osteoprogenitor cells within the same patient from different harvest draws. This variability brings into question the quality of the autogenous harvest. Typically, during aspirations, the best concentration of osteoprogenitor cells is at the first 2-mL draw. The best volume of osteoprogenitor cells is at the 4-mL draw. But clearly, as the patient ages, the actual amount of cells harvested per millimeter diminishes. So, typically when we take autograft or bone marrow aspirates, we look at the quality of the blood draw as an indicator of cells. This clearly is not the case. The quantity of the blood draw represents only the quantity of the red blood cells. It does not necessarily reflect the quantity or quality of the osteoprogenitor cells. Different methods to artificially concentrate the aspirant have been performed using cell filtration and density spin. Both cell filtration models and density spin models concentrate osteoprogenitor cells; however, the density spin typically would concentrate bone marrow and/or aspirate to concentrations of 4.6 times normal with a 99% cell viability and no red blood cell contamination. Autograft can be ideal in that graft has osteogenic, osteoinductive, and osteoconductive properties, but the variability of the osteogenic component—and thereby the osteoinductive component—is not predictable. There is also a potential for substantial donor morbidity in autograft harvests.[13,14]

Osteoinductive Agents

As previously mentioned, an osteoinductive osteobiologic agent has the ability and the bioactivity to "induce" undifferentiated stem cells to become differentiated and produce bone. The gold standard test for inductivity is that when that agent is placed in a non-bone-forming site (such as muscle), ectopic bone would be produced. Various inductive materials are currently available in the orthopedic market. The initial inductive agents were demin-

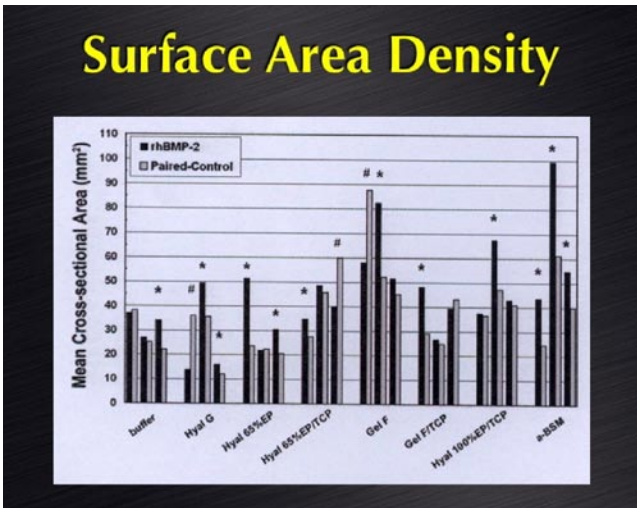

Figure 4. Density of alpha-BSM with rBMP-2 compared with paired control. From: Seeherman HJ, et al.[16] Reprinted with permission from the Journal of Bone & Joint Surgery.

eralized bone. In 1899 Senn utilized decalcified ox bone to help produce bone to fill in defects. In 1930, Levander utilized alcohol extracts of bone and then injected them into muscle, which produced bone. This was one of the first nondefined demonstrations of inductivity. In 1965, Marshall Urist, MD,[10] discovered an agent in demineralized bone matrix (DBM) that stimulated the formation of new bone tissue in wrapped muscle. Dr. Urist coined the protein bone morphogenic protein (BMP) or osteogenic protein (OP). Since then, 2 products have been commercialized as synthetic BMPs. The Stryker Corporation produced OP1 (BMP7) and Sofamor-Danek (Memphis, Tenn) produced Infuse (BMP2). It is important to note that BMPs are a member of the TGF-beta family.[15] This is a 600-million-year-old amino acid sequence. The mechanism of action of commercial BMPs is to serve as osteoinductive agents. BMP7, a product of Stryker Biotech, is an inductive agent in a powdered form. It has approximately a 48-hour lifespan in its physical form (once placed in the body). The cost of BMP7 is approximately $5000 per vial (5 mL). Ideally BMP7 needs complete hemostasis and containment of the graft material to effectively maintain efficacy. BMP is not by itself osteoconductive. Issues of pregnancy, history of cancer, patient age under 18 years, history of bone tumors, and allergies to BMP or collagen are relative contraindications to BMP use.[3,6] BMP2 (Infuse) is a liquid that is mated to a bovine type 1 collagen sponge (BCS). Infuse must be used with the collagen sponge because the BMP binds to the sponge, which is the containment mechanism for the liquid BMP2. Adjuvant BMPs do fulfill an osteoinductive role; however there is no osteogenic component in the BMP nor is there an osteoconductive component in the BMP. As such, BMPs provide 1 component of the ideal triad, and the theory is that by fulfilling this role, providing osteogenic cells, osteoconductivity will develop during the stimulation of the healing cascade by this inductive stimulus.

There are, however, issues with BMP. The cost of a BMP implant is approximately 5 times the cost of plain DBM.[5] Bovine type 1 collagen produces antibodies up to 20% in

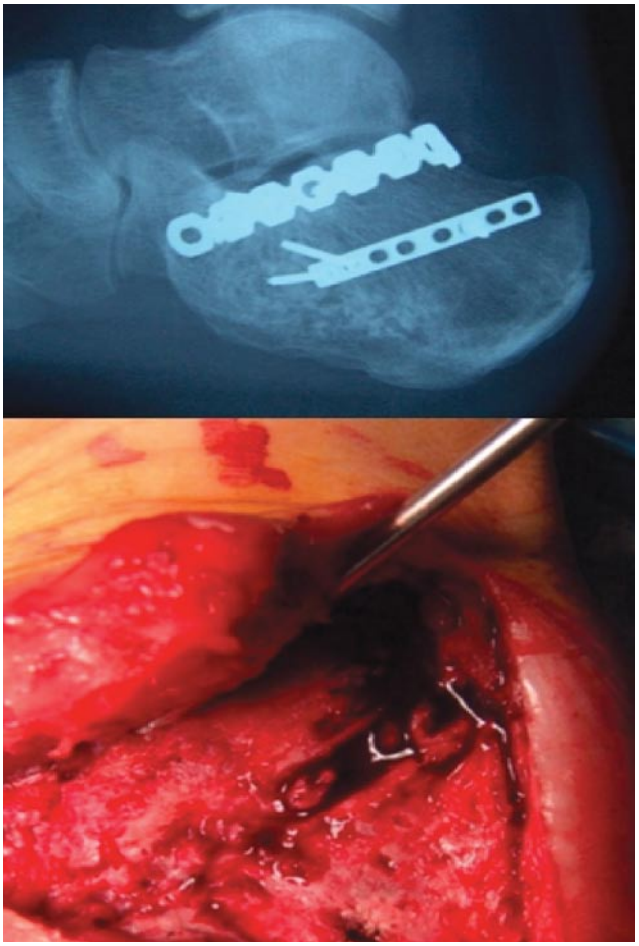

Figure 5. Cortical and cancellous regeneration of calcaneal defect at 6 months after ORIF of calcaneus. Courtesy of Dr. Desai.

subjects.[7] Pregnancy is an issue because of the potential for the mitogenic effect of BMP. Using BMPs in patients who may become pregnant within a year of use is contraindicated. Birth control pills are suggested in this population, with informed consent. There are also potential issues with multiple uses within the same year—that is, antibody effects and efficacy concerns. Both Infuse and OP1 create a temporary inductive effect. BMPs' ability to generate the inductive cascade is dependent on the first 36-48 hours of implantation because of their half-life. According to Hausman and Rinker, "BMP will not work in an environment with no responder cells and is not effective in a devascularized area. BMP also requires an osteoconductive matrix to provide a favorable environment and a scaffold for cell growth."[8] Studies by Aaron[15] have shown that electrical stimulation with the Biomet (EBI) pulsed electromagnetic field device supports a role in chondrogenesis, osteogenesis, and angiogenesis, and an increased TGF-beta of 32% with the use of electrical stimulation. The mechanism of action in electrical stimulation induces angiogenesis, chondrogenesis, and osteogenesis directly. The mechanism of action of electrical stimulation is longer acting and more closely approximates normal osteobiologic gene expression than do BMPs.

BMPs are specific inductive osteobiologic agents that can enhance bone healing. Another osteobiologic inductive agent

is DBM. DBMs are generalized inductive agents that contain more than 1 specific BMP. Unlike OP1 or Infuse, DBM is a general inductive agent. DBM preparations have bioactive BMPs but at a reduced concentration compared with that of isolated BMP preparations. One key aspect of assessing the efficacy of DBMs is bioactivity. DBM preparations are generally given as a percentage of DBM per volume. Bioactivity of the preparation is a better index of efficacy than its concentration.[11] There is no clinical evidence that 100% DBM (which is a crystalline form) is more effective than a more dilute concentration. BMP and DBM bioactivity has not been shown to directly correlate with the concentration of these agents. The Food and Drug Administration (FDA) does not test for bioactivity, just for concentration. Another issue of DBM is the manner in which it is prepared and the disparate carriers used in the mixture. Of all the osteoinductive agents, the synthetic inductive osteobiologics primarily available at this time are Infuse (BMP2), OP1 (BMP7), and the various DBM. There is no study to date that compares and contrasts efficacy and cost of these agents.

Osteoconductive Substances

The third portion of the triad is osteoconductivity. Osteoconductivity is primarily scaffolding needed for growth. When there is direct bony contact, there is no need for an osteoconductive osteobiologic agent; however, if there is a critical gap (>1-cm fracture gap or a gap that cannot spontaneously heal itself), a conductive material is advantageous in bridging that gap and hastening the speed of incorporation. Ideally the osteoconductive surface should have the porosity of cancellous bone, approximately 150-600 microns. From a clinical standpoint, the osteoconductive agent should be resistant to compression. It should also incorporate without weakening, have low morbidity, and be a product that can be drilled through. Autograft has the ideal porosity. Tricortical autograft can resist a compressive load, although there is a potential for harvest morbidity. There is incorporation without weakening and you can drill through it if it is tricortical bone. In allograft corticocancellous chips, the porosity is not controlled and they cannot resist a compressive load. The cost of allograft croutons is minimal, but, for croutons to incorporate, there must be resorption of the allograft itself. In the tibial plateau model, there is a risk of subsidence between 6 and 8 weeks when using allograft corticocancellous chips. The benefit of the osteobiologic osteoconductive agents is that if they are coated with calcium phosphate or hydroxyapatite (HA), there is little need for resorption, and there is an "onlay" healing occurring and, as a result, minimal resorption of graft prior to the deposition of new graft.

Synthetic calcium ceramic osteoconductive materials can be grouped into 2 general categories: calcium sulfate products and calcium phosphate products. Calcium sulfate is crystalline, independent of the rate of resorption, and it is a "true salt." If calcium sulfate is exposed inside the joint, these ions dissolve into Ca^{2+} and $S0_4^{2-}$ ions. These ions do not generate particulate matter in the joint cartilaginous surfaces. Calcium phosphate materials are crystalline, dependent on their rate of resorption. They are a true "ceramic" that does not dissolve within the joint and, as a result, may cause cartilage wear if left exposed in the intracapsular space. Clearly these osteoconductive agents are useful if a "biologic scaffolding" is desired.

Conclusions

The use of osteoinductive, osteogenetic, and osteoconductive agents to aid in healing is not new, but the use of synthetic osteobiologics is recent. The challenge lies not only in understanding what synthetic osteobiologics are available but also when to use which agent. There is also some merit in combining osteobiologics. Seeherman[16,17] has written extensively on the merits of combining BMP and calcium phosphate preparations. The combination of BMP2 and alpha bone substitute material (BSM) composite grafts has shown that the composite inductive agent with an osteobiologic scaffolding not only hastened the rate of fusion but also increased the density of the fusion construct (Figure 4). The use of synthetic osteobiologics in a coordinated and combined fashion shows promise in the appropriate use of grafting products in the orthopedist's armamentarium (Figure 5).

The introduction of osteobiologics to hasten union is the next challenge in fracture care. The use of various locking plates has aided in our ability to stabilize fracture constructs. The goal of fracture union and ultimate fracture stability lies in the body's ability to heal and our ability to accelerate this process.

Author's Disclosure Statement

Dr. Desai discloses that he is a consultant to Biomet, Inc., and Zimmer, Inc.

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