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AOA Critical Issues

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Introduction

Improvements in our understanding of biological and biomechanical aspects of connective-tissue degeneration, healing, and repair have revealed the potential to develop novel approaches to tissue repair and regeneration. New knowledge about the basic cellular and molecular processes that govern tissue repair has emerged from the fields of cell biology, molecular biology, and molecular genetics. Ongoing developments in related fields, such as biomaterials and stem cells, have also suggested new ways to attack old problems in orthopaedics.

This symposium brought together experts to address several important areas: (1) the use of local osteoinductive factors for spine fusion, (2) systemic agents and mesenchymal stromal cells to manipulate bone formation and remodeling, and (3) the use of scaffolds and platelet-derived factors for ligament-healing. We discuss biologic approaches to tissue-healing and regeneration, ranging from experimental techniques that are

currently in preclinical testing to techniques that are currently in use. The basic principles related to tissue-healing and regeneration presented here will provide the basis for future studies in tissue engineering and related fields.

Current Status of Osteoinductive Agents for Spine Fusion

Spine arthrodesis is one of the most challenging applications for bone graft materials and bone-healing. The nonunion rate associated with arthrodesis for management of degenerative lumbar spine disease ranges from 10% to 45%^{1,2}. The combination of biologic and environmental factors makes the achievement of spine fusion sensitive to systemic issues that inhibit bone-healing, such as nonsteroidal anti-inflammatory drugs, corticosteroids, and nicotine.

Bone graft substitutes are associated with one or more of four potential healing mechanisms³. An osteogenic material, by

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definition, has live osteoblasts that are capable of making bone matrix, which mineralizes normally. Osteoconductivity is the ability of a material to serve as a scaffold to support new bone ingrowth and/or ongrowth and can be influenced by the three-dimensional macrostructure as well as features such as microporosity.

Osteoinductivity is the unique ability of a protein, when implanted in an ectopic location, to generate *de novo* bone formation via recruitment and differentiation of progenitor cells, development of blood supply, and formation of mature bone-marrow elements. The prototypical osteoinductive agent is bone morphogenetic protein (BMP), which is present in trace amounts in all bone but is only active in bone that has been demineralized. Lastly, some proteins are osteopromotive; that is, they can accelerate or facilitate bone formation that is already occurring but they are not capable of initiating *de novo* bone formation ectopically.

The biologic healing milieu in the posterolateral aspect of the spine is such that purely osteoconductive materials usually are not adequate to serve as stand-alone bone graft substitutes, with the possible exception being when they are used during arthrodesis in patients with adolescent idiopathic scoliosis⁴. Data from animal studies would suggest that a bone graft substitute with some level of osteoinductivity is most effective for posterolateral spinal arthrodesis because it can combine the creeping substitution process of bone ingrowth from the decorticated host bone, with recruitment and differentiation of additional progenitor cells from the surrounding soft tissue adding a centripetal pattern of bone-healing^{5,6}. Specific BMPs (BMP-2, 4, 6, 7, and 9) have demonstrated the potential for varying degrees of osteoinductivity⁷.

Demineralized bone matrix is generated by numerous processors and often combined with various carrier materials to improve the handling characteristics of the final material. Combinations with carriers, sterilization methodology, and many other variables can lessen or eliminate the osteoinductivity⁸⁻¹¹. Because the historic regulatory position has been that these products constitute minimally manipulated human tissues, none of the currently available demineralized bone matrix products have undergone extensive study in human clinical trials. Some demineralized bone matrix preparations have undergone more rigorous testing in animal models (e.g., rabbits and monkeys) and have been shown to have the ability to enhance the healing success of autologous bone graft used in animals undergoing posterolateral lumbar arthrodesis, but these preparations cannot suffice on their own as a stand-alone material^{12,13}. Because of the inability of any demineralized bone matrix to serve as a stand-alone substitute for challenging spinal arthrodesis situations, much of the focus on osteoinductive agents for spine fusions has centered on BMPs.

Marshall Urist purified BMP extracts from multiple species and showed their consistent ability to demonstrate osteoinductive properties. Recombinant human BMP-7 (rhBMP-7) (OP-1 [osteogenic protein-1] Putty; Olympus Biotech, Hopkinton, Massachusetts) was granted humanitarian device exemption approval in 2004 for use in revision posterolateral

lumbar spine fusion when autologous bone and bone marrow harvest are either not feasible or not expected to promote fusion. Despite multiple attempts, this device has failed to gain full premarket approval for spine or trauma by the U.S. Food and Drug Administration (FDA). The primary spine trial used a noninstrumented posterolateral spine fusion model that presents challenges in obtaining fusion. In some studies, the fusion rates obtained with rhBMP-7 were neither superior to the rates obtained with use of autograft nor comparable with the rates obtained with use of other rhBMPs, which may be because the concentration of rhBMP-7 (0.8 mg/cc) in the OP-1 implant may be below the necessary threshold for consistent bone induction in primates¹⁴.

The lack of full premarket approval in the decade since the OP-1 implant first received humanitarian device exemption approval in 2002 for use in trauma patients suggests that a clear demonstration of clinical effectiveness for this product has been lacking. For example, in a study of thirty-six patients who underwent instrumented single-level posterolateral fusion, the fusion rate was 63% in the OP-1 group and 67% in the control group¹⁵. Another study reported that radiographic evidence of a solid fusion was present in only eleven (69%) of sixteen patients in whom OP-1 putty was used¹⁶.

RhBMP-2 (INFUSE; Medtronic, Memphis, Tennessee) was granted premarket approval in 2002 for anterior lumbar interbody spinal fusion in conjunction with threaded fusion cages¹⁷. The INFUSE device typically delivers 6 to 12 mg of rhBMP-2 (1.5 mg/mL) on an absorbable bovine type-I collagen sponge placed inside a metal interbody fusion cage. This particular carrier was optimized for use in the interbody space inside a structural element (cage), and higher doses (up to 40 mg) with composite carriers composed of collagen and ceramic have been optimized for use in posterolateral spinal fusion but have not yet gained FDA approval¹⁸⁻²¹. Achievement of radiographic evidence of successful interbody fusion with this technique can be as high as 98% on the basis of computed tomographic (CT) scan evaluation, with superiority over autogenous bone graft²². Moreover, recent data based on review of a large administrative database²³⁻²⁵ suggest that INFUSE can be effective in patients who are older than sixty years and in patients who smoke and that INFUSE does not result in increased complications (except when used in high doses in the cervical spine).

It has been estimated that as much as 85% of the usage of INFUSE may be in off-label, or physician-directed, applications²⁶. While some of these applications represent minor differences from the approved indication for anterior lumbar interbody fusion (e.g., use with a different type of cage or insertion via a different surgical approach), others are a more substantial departure (e.g., cervical interbody fusion, posterolateral spinal fusion, and long fusions for deformity with use of substantially higher doses). The majority of side effects have been local ones (e.g., fluid collection, radiculitis, transient osteoclastic activity, and bone formation in the surgical track) and are largely related to improper usage of the INFUSE device in these alternative locations^{27,28}. Unfortunately, the

manufacturer is precluded from education on proper usage in off-label applications, so the correct and safe techniques for these alternative uses travel slowly by word of mouth and, eventually, through inclusion in physician-directed publications. Many of the complications can be related to the use of too much INFUSE, overpacking of the sponge, use near aggressively decorticated cancellous bone and/or bone marrow, and use near excessive bleeding, with the resultant hematoma increasing the potential for adjacent ectopic bone formation²⁹. The anterior aspect of the cervical spine seems particularly prone to edema and the formation of a seroma or hematoma when excessive amounts of INFUSE are used³⁰. It should be noted that, in July of 2008, the FDA issued a public health notification regarding potentially life-threatening complications due to swelling in the anterior aspect of the neck as a result of rhBMP use in the anterior aspect of the cervical spine³¹. The incidence of these local complications is substantially lower when the correct dosage of INFUSE is utilized, and a clinical trial is under way to confirm the safety profile³².

Moving forward, there will be several challenges to the full utilization of the potential benefit of osteoinductive agents for spine fusion. First, the rhBMPs must achieve expanded indications with specific dose and carrier formulations that provide location-specific safe and consistent delivery with minimal local side effects. Second, the osteoinductive capabilities of other proteins currently under investigation must be proven as being equal or better to that of rhBMPs before it makes sense to commercialize them. Lastly, an ideal next step would be to identify a synthetic small molecule that would truly be osteoinductive and that would afford flexibility for delivery options and have an easier manufacturing process as compared with today's expensive recombinant osteoinductive proteins. Once consistent osteoinductive effectiveness can be reproduced, safety and cost remain the key obstacles to the ultimate evolution away from autogenous bone graft as the gold standard.

The Potential Role of Parathyroid Hormone (PTH) as a Systemic Agent for the Enhancement of Skeletal Repair and Implant Fixation

Parathyroid hormone (PTH) is a polypeptide containing eighty-four amino acids. The function of PTH is to maintain mineral homeostasis by increasing gastrointestinal calcium absorption, increasing renal calcium and phosphate reabsorption, liberating calcium from the skeleton in response to systemic demands, and participating in the regulation of vitamin-D metabolism³³. Studies have shown that, whereas continuous exposure to PTH leads to an increase in osteoclastic activity and results in bone resorption, intermittent exposure stimulates osteoblasts and results in an increase in bone formation^{34,35}. Normal secretion of PTH is regulated by physiological demand as dictated by the serum level of ionized calcium. Although these levels are constantly in flux, the continuous nature of this exposure leads to osteoclastic activity and, consequently, bone resorption. However, when rhPTH (1-34) is administered therapeutically, the once-daily bolus administration creates a pulsatile exposure and leads to increased osteogenesis^{34,35}.

Studies in models of fracture-healing have demonstrated that rhPTH (1-34), the active fragment of this polypeptide, consistently enhances the stiffness and strength of experimental fractures during the healing process³⁶⁻³⁹. In an investigation by Alkhiary et al.³⁸ the effects of rhPTH (1-34) (teriparatide) on fracture-healing was studied in 270 rats that had undergone standard closed femoral fractures and received doses of teriparatide that are similar to those that are effective in the treatment of osteoporosis in postmenopausal women. Daily systemic administration of both a 5 mg/kg and a 30 mg/kg per-day dose enhanced fracture-healing by increasing bone mineral density, bone mineral content, and total osseous tissue volume.

While studies such as these clearly demonstrate the potential enhancement of bone repair with teriparatide, the mechanism through which it exerts its effect has not been fully elucidated. Kakar et al.⁴⁰ showed that teriparatide primarily enhanced the early stages of endochondral bone repair by promoting chondrocyte recruitment and differentiation. This resulted in the formation of a larger callus cross-sectional area, length, and total volume compared with those in controls. Molecular analysis of the expression of extracellular matrix genes associated with chondrogenesis and osteogenesis showed a threefold greater increase in chondrogenesis relative to osteogenesis in the rhPTH-treated fractures. More recent work from our laboratory (unpublished data) shows that the expression of matrix metalloproteinase occurs earlier in fractures treated with teriparatide, suggesting an enhancement of cartilage turnover and transition from calcified cartilage to bone.

On the basis of the results of these preclinical studies, some clinical investigators have used teriparatide in the treatment of patients with fractures and have subsequently reported individual cases or small case series⁴¹⁻⁴⁴. However, only one randomized trial has been conducted to address this question. Postmenopausal women who had sustained a dorsally angulated distal radial fracture in need of closed reduction and nonoperative treatment were randomly assigned to eight weeks of once-daily injections of placebo, teriparatide (20 µg), or teriparatide (40 µg), within ten days of fracture. The results showed that the median time from fracture to first radiographic evidence of complete cortical bridging in three of four cortices was 9.1, 7.4, and 8.8 weeks for placebo, teriparatide (20 µg), and teriparatide (40 µg), respectively ($p = 0.015$), and that the ultimate time to healing was shorter in the teriparatide (20 µg) group than in the placebo group ($p = 0.006$). The report concluded that the shortened time to healing suggests that fracture repair can be accelerated by teriparatide, but that these results require further investigation⁴⁵.

Use of Concentrated Autologous Bone-Marrow Stem Cells for the Enhancement of Skeletal Repair and for the Treatment of Osteonecrosis

Several investigators have confirmed the osteogenic capacity of the stem cells that reside in autologous bone marrow⁴⁶⁻⁴⁹. Indeed, Connolly et al.⁵⁰ demonstrated a positive correlation between the osteogenic capacity and the cell concentration of bone marrow, and this led to the use of enhanced osteogenic bone-marrow

preparations for the treatment of nonunions^{51,52}. Hernigou et al.⁵³ studied the influence of the number and concentration of progenitor cells in sixty patients with noninfected nonunions. Bone marrow was harvested from the iliac crest, concentrated, and injected. At four months after treatment, union was obtained in fifty-three of sixty patients and there was a positive correlation between the volume of mineralized callus and the number and concentration of colony-forming units in culture. The seven patients who did not heal had significantly lower numbers and concentrations of colony-forming units.

In addition to their use in the treatment of fractures, bone-marrow stem cells have been investigated in the treatment of osteonecrosis of the femoral head, which has been estimated to be the diagnosis in up to 12% of patients who undergo total hip arthroplasty⁵⁴. Treatment options for patients who do not have flattening of the femoral head or “pre-collapse” have been associated with a mixture of reported results. Gangji et al.⁵⁵ and Hernigou et al.^{56,57} have shown that concentrated bone-marrow stem cells provide an effective autologous graft for augmentation of the potential beneficial effects of core decompression. Further analysis and the initiation of randomized controlled trials in which core decompression with stem cells is compared with core decompression alone will be required before the success of this intervention can be determined.

Gangji et al. performed a five-year follow-up on a prospective controlled study that was previously reported⁵⁸. The investigators studied nineteen patients (twenty-four hips) who had early-stage osteonecrosis of the femoral head. The hips were allocated to either core decompression only or core decompression and implantation of bone-marrow cells. The patients as well as the investigators were blinded with respect to treatment group assignment. The primary outcomes were clinical symptoms and disease progression. The results showed that implantation of bone-marrow cells resulted in a significant reduction in pain and joint symptoms and reduced the incidence of femoral head fracture and collapse. At sixty months, eight of the eleven hips in the control group had deteriorated, whereas only three of the thirteen hips in the group treated with bone-marrow cells had deteriorated. Survival analysis showed a significant difference in the time to failure between the two groups at sixty months.

One of the concerns regarding the use of bone-marrow cells relates to the age of the patient. It is well known that the number of bone-marrow progenitor cells decreases with age, and this has led to the belief that efficacy of bone-marrow grafting may decline with age⁵⁹. However, at this time no studies have been conducted to test this hypothesis and very few patients over the age of sixty years have been subjected to this treatment.

Use of Scaffolds and Platelet-Rich Plasma for Ligament-Healing

Recently, a series of studies were performed to elucidate reasons for the poor healing potential of the anterior cruciate ligament (ACL). An initial study of histological changes in the ruptured ACL found that the human ACL did not have any tissue in the

gap between the ligament remnants and that, as a result of that failure of filling of the wound, myofibroblasts were able to migrate over the ends of the torn ligament and contribute to ligament retraction and impaired healing⁶⁰. In extra-articular tissues that heal, such as the medial collateral ligament (MCL), a fibrin clot forms in the wound site and is invaded by fibroblasts that gradually replace the fibrin with collagen fibers. This has been demonstrated to be instrumental in the healing process in both tendon and ligament^{61,62}. However, it has been demonstrated that fibrin clot does not form in the intra-articular milieu, probably because of the presence of fibrinolytic enzymes in synovial fluid^{63,64}. One reason, then, for the failure of the human ACL to heal is the lack of a bridging scaffold persisting between the ruptured ligament ends long enough for the surrounding fibroblasts to invade and remodel it into fibrovascular tissue.

If it is this lack of an early bridging scaffold between the ends of the ACL that may be impeding the ability of the ACL to heal, then perhaps placement of a substitute scaffold in the ACL wound site could stimulate healing of the ligament. At a minimum, this scaffold would need to have resistance to premature enzymatic degradation within the synovial environment. This requirement makes fibrin-based scaffolds of less potential interest, as the fibrin-based natural clot is predisposed to premature dissolution in the joint environment.

In addition to a simple structural scaffold, it is likely that, to stimulate successful ACL-healing, additional biologic stimuli may need to be added. Individual growth factors help stimulate ACL cell migration and proliferation and collagen production^{65,66}; however, application of these factors can be expensive and determinations of appropriate concentrations and timing of delivery can be complex. A simpler alternative is to work initially with platelets, which are cells that act as a native growth-factor delivery system within the fibrin clot in wound sites of the MCL and other successfully healing tissues. Platelets start the wound-healing cascade and recruit multiple other cell types to the wound, with expression of over 1000 genes. Thus, harnessing these cells as the biologic stimulus for an implanted scaffold seems a logical and translational step to improving wound-healing in the ACL. However, successful use of these cells may require a careful activation of the platelets so that they will release their growth factors in a physiological way.

Collagen is a naturally occurring protein (and the main extracellular matrix protein of the ACL). In addition to its structural properties, it also has the ability to combine with fibrin to make a plasmin-resistant copolymer⁶⁷. Thus, a collagen-fibrin scaffold could potentially have an improved ability to resist premature degradation within the joint. Collagen also is a substrate that encourages cell migration within the scaffold⁶⁸. Lastly, collagen is a known activator of platelets and results in a more physiological and sustained release of growth factors from platelets in comparison with that seen with thrombin-based techniques^{69,70}.

Thus, one possible biomaterial that could be used for intra-articular healing is a collagen-platelet composite scaffold. The collagen provides mechanical and enzymatic stability, while the

platelets deliver a biologic stimulus^{71,72}. In vivo analysis was done with use of a model in which a central axial slit is made in the center of the ACL⁷³. This slit only transects approximately 60% of the ACL fibers and is mechanically stabilized on either side by the remaining ACL fascicles. Thus, it provides an excellent model for evaluating the biology of the response to the biomaterial without the complicating factors of variation in mechanical stability that would be associated with a complete transection-repair model. Even with these optimal conditions, we verified that the central defect remained open and failed to heal in the ACL without treatment. Using this model, we were able to demonstrate that placement of a collagen-platelet scaffold in the central defect could stimulate histologic healing of the defect⁷⁴. In addition, we also found that placement of a collagen-platelet composite would stimulate biomechanical healing of the ACL as well, thus not only closing the defect with living scar, but with a scar that could support load⁷⁵.

With these promising results from the central defect model, we then progressed to a second model of complete ACL transection. In this model, the ACL was transected at the junction between the middle and proximal thirds, which is the most common location for clinical ACL rupture. A suture repair was performed and then a collagen-platelet scaffold was placed in the gap between the two severed ends of the ligament (a technique called the *bio-enhanced suture repair*). Using this model, we found that placement of a collagen-platelet scaffold in the wound site of the ACL could stimulate both histologic and biomechanical healing of the ligament⁷⁶ and that, by three months, the strength of the ACL treated with a bio-enhanced repair technique was similar to that previously reported for ACL reconstruction in similar animal models⁷⁷. Interestingly enough, when either a collagen scaffold alone⁷⁸ or a platelet solution alone⁷⁹ was used as the bio-enhancement, there was no statistically significant improvement over suture repair alone.

There have been many recent advances in ACL reconstructive surgery, including graft types, fixation methods, and refined surgical techniques. However, studies reporting Level-I or Level-II evidence in the investigation of the effects of these

advances have not yet been able to show a significant improvement in outcomes for patients. The persisting high rates of graft failure in adolescents and posttraumatic osteoarthritis after an ACL tear make one wonder whether an entirely new approach to this injury, such as repairing the ACL rather than replacing it, will someday be warranted. Clearly, ACL repair is an approach that is still in its infancy, with a great deal of room for improvement, and additional preclinical development coupled with initial clinical trials are needed to investigate whether this nascent technology will have a positive effect on the outcomes for patients with ACL injuries. ■

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