Clinical Applications of Bioactive Factors in Sports Medicine

Current Concepts and Future Trends

Michael J. Angel, MD, Nicholas A. Sgaglione, MD,† and Daniel A. Grande, PhD‡*

Abstract: The ability to biologically manipulate musculoskeletal healing and augment bone and soft tissue repair and regeneration holds great promise. Advances in the basic science study and clinical application of bioactive proteins and growth factors continues to evolve. Improvement in the surgical resurfacing of articular cartilage defects and tendon and ligament repair through the addition of bioactive polypeptides is currently underway. The purpose of this article is to review the present array of biologically active materials that may be clinically applicable in sports medicine and arthroscopy. Mechanisms for biologic augmentation of tissue repair and regeneration will be discussed. Current limitations and future considerations will be reviewed particularly as they relate to practical clinical approaches.

Key Words: bioactive proteins, growth factors, biologic augmentation, soft tissue repair, clinical approaches

(*Sports Med Arthrosc Rev* 2006;14:138–145)

Biologic advances in the approach to soft tissue surgery in orthopedics have recently attracted much basic science and clinical attention. Current trends suggest that the future of soft tissue repair will in large part be biologically based. Treatment strategies will most likely focus on manipulating and accelerating the biology of healing and augmenting the mechanical strength of repairs with exogenous biologic growth factors. Clinical applications include biologic approaches to focal articular cartilage defects as well as the acceleration of soft tissue healing to bone required in knee ligament reconstruction, tenodesis procedures, rotator cuff repair, and glenohumeral capsulorrhaphy. In addition, as meniscus preservation is increasingly emphasized, the potential for broadening meniscus repair methods in avascular zones or biologically challenged complex tear patterns is expanding. Ultimately, the improvement on tissue restoration and acceleration of the repair process will expedite recovery, rehabilitation, and return patients to functional activities more quickly.

Soft tissue repair is based upon the interaction of several biologic and physiologic components: a structural base onto which cells and/or tissue can be introduced and implanted into the pathologic site (ie, scaffold), the addition of a cell line and responsive cellular environment to promote extracellular matrix production, and a bioactive stimulus for tissue maturation and response to mechanical loading[1,2] (Fig. 1). This article will focus on the practical applications of bioactive growth factors and other biomaterials that may be used to augment the repair process. Specific growth factors as well as their current and future applications in the field of sports medicine will be reviewed.

DISCUSSION

Growth Factors

Bioactive factors are essential proteins released at the site of injury by repair/inflammatory cells that then bind to receptors influencing critical cellular functions. Many of these bioactive proteins are capable of an anabolic response resulting in cell proliferation and differentiation (mitogenic response), cell migration (chemotaxis), and matrix synthesis. In addition, angiogenesis may be stimulated, inducing the growth of new blood vessels. Bioactive factors may also exert a catabolic effect resulting in local cell apoptosis, matrix suppression, and decreased protein synthesis. Various other polypeptide factors may play a role including cytokines which are extracellular proteins that mediate cell to cell signaling inducing structural changes to mesenchymal tissue. Tumor necrosis factor, interleukin-1), and interferon are examples of cytokines.

Growth factors may be described based on their receptor-mediated response through the tyrosine kinases, small G-protein–associated receptors, or serine/threonine kinases and have a wide range of effect on musculoskeletal tissues. The transforming growth factor-β (TGF-β) superfamily consists of over 100 members with numerous anabolic effects on all types of musculoskeletal tissue (Table 1). Bone morphogenic proteins (BMPs) are released by osteoprogenitor cells and typically improve

From the *Department of Orthopaedic Surgery, Long Island Jewish Medical Center; † Department of Orthopaedic Surgery, Long Island Jewish Medical Center, North Shore University Hospital Health System; and ‡North Shore/Long Island Jewish Research Institute, Manhasset, NY.

Reprints: Dr Nicholas A. Sgaglione, MD, Orthopaedic Associates of Manhasset, 600 Northern Blvd, Great Neck, NY 11021 (e-mail: nas@optonline.net).

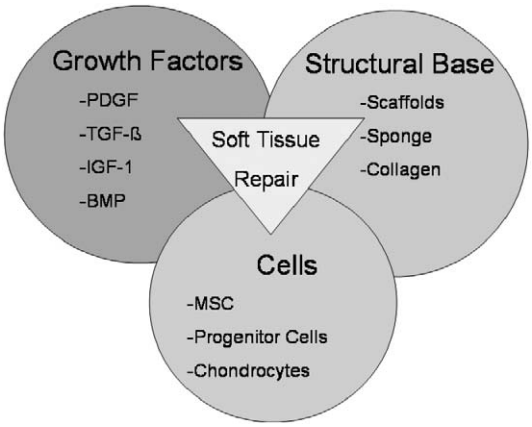

FIGURE 1. Illustration of the 3 components of soft tissue repair: cells, scaffold, and factors.

bone growth. The BMPs are members of the TGF-β superfamily. Platelet-derived growth factor (PDGF) is found in high concentration localized in the α granules of circulating platelets and endothelial cells and has been shown to play a role in chemotaxis, angiogenesis, and mitogenic mechanisms. PDGF exists as 3 distinct isomers (AA, AB, and BB) based upon integral polypeptide chain constituents. Insulinlike growth factor-1 and -2 (IGF-1 and -2) can be synthesized by variable cell types and exert a broad anabolic cellular response. Basic fibroblast growth factor (bFGF) consists of at least 19 different subtypes and plays a primary role in the early development and differentiation of musculoskeletal and nervous system tissue as well pathologic tissue undergoing repair.[3] Epidermal growth factor (EGF) has proliferative effects on cells of mesoderm and ectoderm origin especially fibroblasts.[3] Growth and differentiation factor-5 (GDF-5 or BMP-14), another member of the TGF-β superfamily plays a role in chondrocyte, fibroblast, and mesenchymal cell expansion.

Basic Science of Healing

Tissue repair and healing relies on vascularity and cellular migration. After injury, damaged cells undergo apoptosis.[3] Blood and interstitial fluid escapes from traumatized blood vessels leading to the formation of a hematoma. The hematoma traps platelets, which upon activation release growth factors and cytokines with chemotactic and mitogenic properties. Pluripotent stem cells and inflammatory cells form a dense fibrous scaffold that serves as a volume-stable substrate for subsequent neocellular proliferation. Further remodeling proceeds with increased vascularization and granulation tissue formation.

Essential to the repair process is the intrinsic cell line and its environment. Different cell types may respond in various manners to specific factors whereas certain cell lines such as fibroblasts may respond differently to the same growth factor depending upon the particular tissue type.[4,5] It follows then that progenitor and mesenchymal stem cells are an optimal target for manipulation of healing[6,7] because of their pluripotent capacity. The application of a bioactive peptide or growth factor that could accurately and specifically control the differentiation lineage and maturation of mesenchymal stem cells and reliably enhance specific cell phenotypic expression would have great potential in soft tissue repair.

CLINICAL APPLICATIONS

Bone to Bone Healing

Fracture repair augmented by the addition of growth factors is arguably the foundation of tissue engineering in orthopedic surgery. Recent published work has described the application of various bioactive factors including BMP, TGF-β, FGF, PDGF, and IGF.[8] Currently 2 proprietary BMP preparations have been released for clinical use; recombinant BMP-2 (rhBMP-2: Infuse, Medtronic Sofamor Danek, Minneapolis, MN) and BMP-7 (osteogenic protein-1 or OP-1, Stryker Biotech, Hopkinton, MA). Both have been approved for use in spinal fusion.[9–14] Several animal model studies have demonstrated the effectiveness of BMP-2 in fracture repair. Lee and coworkers[15] demonstrated 85% healing at 2 weeks and 95% to 100% healing at 4 weeks in murine bone defects treated with muscle cells engineered to express BMP-2. In contrast, controls exhibited 30% to 40% healing at 4 weeks.[15]

TABLE 1. Growth Factors and Their Effects on Musculoskeletal Tissues

	Skeletal Muscle	Hyaline Cartilage	Meniscus	Ligament/tendon	Bone
BMP-2	—	⇧	⇧	—	⇧
BMP-7	—	—	—	—	⇧
BMP-12	—	—	—	⇧	—
GDF-5	—	—	—	⇧	⇩⇧
TGF-α	⇧	—	⇧	⇩	—
TGF-β	⇩⇧	⇧	⇩⇧	⇩⇧	⇧
PDGF	—	—	⇧	⇩⇧	⇩
IGF-1	⇧	⇧	⇩⇧	⇩⇧	⇧
bFGF	⇧	⇧	⇧	⇩⇧	⇧
EGF	⇩	⇧	⇧	⇧	—

⇧ enhances repair. ⇩ inhibits repair. ⇩⇧ exhibits both.

In 2002, the "BMP-2 Evaluation in Surgery for Tibial Trauma" study reported on a 44% reduction in the risk of failure as defined by lack of union in open tibial fractures when treated with intramedullary fixation and BMP-2 infused collagen sponges. A dose-dependent response was observed with higher doses undergoing fewer secondary interventions.[16] BMP-2 was also reported to be clinically effective in 46 patients undergoing anterior interbody fusion with BMP-2 treated allograft dowels when compared with patients undergoing fusion with iliac autograft.[17] Other basic science work has been published using LIM mineralization protein (LMP-1), an anabolic growth factor. Boden et al[18] demonstrated successful fusion in 100% of cases in a murine model undergoing posterior spinal fusion with marrow cells transfected with LMP-1. This success was noted in contrast to 0% healing in the control specimens.[18] LMP-1 has been postulated to act as a cytokine further inducing multiple BMPs.[19]

Bioactive factors may also exert an inhibitory effect on healing. A recent study by Ranly et al[20] determined that PDGF may have an inhibitory effect on the osteoinductive properties of demineralized bone matrix in muscle. This illustrates the need for more clinical study of various exogenous growth factors because the same study also showed that platelet-rich plasma may inhibit osteoinduction. Bone to bone healing may not be solely dependent on the delivery of platelets and inflammatory cells. It is likely a complex array of factors and feedback mechanisms contribute to osteoinduction.

Anterior cruciate ligament reconstruction (ACLR) using bone-patella-tendon-bone grafts is commonly performed; however, there is limited available data on the use of bioactive factors to enhance the bone-to-bone interface of the tibial and femoral tunnels. ACLRs typically fail at the bone-tendon interface or midsubstance during the critical "ligamentization" period. It is during this time period where the transplanted graft undergoes a period of weakness and is susceptible to rupture.[21]

Proximal tibial osteotomy has recently generated a resurgence of interest among clinicians treating active aging athletes with unicompartmental arthrosis and who are not candidates for arthroplasty. The use of BMP-2 and OP-1 in bone defects and repair may ultimately hold clinical promise in osteotomy surgery. Reliable bony healing would reduce the morbidity associated with delayed union and nonunion, and would potentially expedite recovery and postoperative rehabilitation allowing patients to ambulate and return to activities sooner.

Bone to Tendon Healing

The current literature does not conclusively define the mechanism of healing of a tendon graft in a bone tunnel. Bone to tendon healing may occur in a direct or indirect manner. Rodeo[22] demonstrated indirect healing in the study of an extra-articular model in dogs. They showed early cellular migration of collagen fibrils resembling Sharpey fibers with continued bone remodeling resulting in bone in-growth and mineralization over time.[22] Other authors, however, have shown that healing may occur in a direct manner with fibrocartilage between the tendon and bone.[23,24] This suggests that multiple factors are dynamically involved including the presence of multiple growth factors, cytokines, and a changing cellular environment.

Rodeo[25] also reported on the use of BMP-2 to enhance tendon to bone healing in ACLR by augmentation of bone in-growth at the tendon-bone interface. Histologic and biomechanical data showed that BMP-2 improved the healing of transplanted digital extensor tendons in bone tunnels in the proximal tibia of dogs.[25] In the BMP-2 treated specimens, a narrower bone-tendon interface zone was noted histologically indicating more optimal graft incorporation. It was also noted that tendon pullout occurred at the bone-tendon interface in all samples at 2 weeks, whereas at 4 and 8 weeks the controls continued to fail in the same manner. The BMP-2 treated specimens in contrast, failed by rupture at the midsubstance or the musculotendinous junction. Anderson[26] reported on the use of an exogenous complex bioactive agent (Bone Protein, Sulzer Biologics, Wheat Ridge, CO) to enhance healing of semitendinosus tendon grafts in 70 rabbit models. The treated specimens exhibited a 65% increase in tensile strength compared with controls. The proprietary bone protein product was assayed using immunoblot analysis and was noted to contain BMP-2 to 7, TGF-β -1 to 3, and FGF-1. Further laboratory work examined the use of semitendinosus tendon grafts infected with adenovirus-BMP-2 in rabbits.[27] Improvement in construct stiffness was noted in the BMP-2 treated specimen. The overall stiffness was 73% higher (29.0 N/mm compared with 16.7 N/mm) in the BMP-2 gene-enhanced specimens compared with the controls. Ultimate load to failure was also improved by 142% (108.8 N compared with 45.0 N) in the BMP-2 enhanced tendons.

Mihelic[28] reported on the addition of OP-1 to a collagen sponge with insertion into the bone tunnel of 30 sheep undergoing ACLR using a peroneus tertius autograft. Histologic analysis revealed improvement in bone formation at the bone-tendon interface whereas biomechanical testing demonstrated pull-out strength of 368 N in the OP-1 treated specimens versus 214 N in controls. In the OP-1 treated ACL specimens, most tears occurred midsubstance whereas control ligament specimens most often failed at the bone-tunnel interface.

The augmentation of tendon-to-bone healing may play a critical role in the treatment of ACLRs, rotator cuff repairs, and collateral ligament reconstructions. Early basic science outcomes associated with the addition of BMP-2 and OP-1 are promising and suggest broadening potential for future clinical applications.

Tendon-to-Tendon Healing

The mechanism of tendon healing remains incompletely defined; however, recent work indicates that GDF-5 may play a role. In a study of mouse-tail tendon

formation, the inhibition of GDF-5 led to morphologic alterations of type 1 collagen fibrils.[29] There was a 17% increase in medium-size collagen fibrils compared with large-size fibrils and a 33% increase in irregularity of shape. Chabra et al[30] demonstrated that Achilles tendons from 8-week-old male GDF-5 −/− mice exhibited a short-term delay of 1 to 2 weeks in the healing process compared with phenotypically normal controls. The GDF-5 deficient tendons also contained significantly more fat within the repair tissue, and were significantly weaker than controls at 5 weeks, but strength differences were no longer detectable by 12 weeks. Aspenberg and coworkers demonstrated that GDF-5 and -6 implanted onto collagen sponges in the transected and denervated Achilles tendons of 66 rats, exhibited improved tensile strength at 2 weeks when compared with controls (collagen sponges alone).[31] This data supports that GDF-5 plays a significant role in tendon-to tendon healing and may be used to enhance and augment repair.

Recently, laboratory data has demonstrated the ability to improve tendon repair by using an IGF-1 and PDGF-2 gene-enhanced tissue engineered platform to augment collagen synthesis and promote mitosis. Rat tendon fibroblasts were transduced with factors in vitro and inserted into rotator cuff tears repaired in vivo with a polyglycolic acid collagen scaffold. The tissues were then harvested and examined at 6 weeks postoperatively. The models exhibited a 10-fold increase in collagen synthesis compared with controls as identified by tridiated proline analysis (Dines, personal communication).

BMP-12 (or GDF-7) is another BMP subtype. It has been studied by Lou et al,[31] who demonstrated a repair construct that did not cause differentiation of mesenchymal cells, as would be expected with the addition of BMP-2. The authors showed a paucity of production of alkaline phosphatase (a marker of cell differentiation by osteoblasts) associated with the use of BMP-12 compared with BMP-2 thus suggesting that BMP-12 has a different mechanism of action from BMP-2. The same author studied the effects of adenovirus-mediated gene transfer of BMP-12 into chicken tendon cells. They demonstrated a proliferation of type 1 collagen synthesis that resulted in a 2-fold increase in tensile strength and stiffness.[32] Further studies of BMP-12 will provide a better understanding of the mechanisms of tendon healing and improve our ability to more precisely target repairs.

The long-term goal of tendon repair is to develop biologically active, easily deliverable methods with which to enhance growth factor expression and in turn augment repair. Tissue augmentation patches comprised of porcine small-intestine submucosa, bovine, equine collagen, and human allograft dermis may hold promise in cases of massive tears or attenuated tissue associated with rotator cuff, Achilles, and knee extensor ruptures. More importantly, they may provide an excellent platform for the addition and insertion of bioactive factors in the future.

Ligament Healing

After ACL injuries, in vivo studies have demonstrated that certain growth factors and cytokines are elevated.[33,34] PDGF and TGF-β are found to be elevated in the first 3 to 7 days after injury, whereas levels of TGF-β, IGF-1, and IGF-2 continued to be found at elevated levels for up to 3 weeks after injury to ligaments. Studies investigating the effects of bFGF, PDGF, VEGF, and TGF-β on fibroblasts derived from the medial collateral ligament (MCL) and the ACL have shown an increase in cell proliferation, type 1 collagen and proteoglycan synthesis.[35,36] Batten et al,[37] in a study of injury sites of murine MCLs implanted with PDGF versus collagen controls found a dose dependent effect with the use of PDGF resulting in a 90% increase in stiffness in healing. PDGF also has been shown to be extensively involved in division and cellular migration of fibroblasts.[38] Scherping et al[21] studied the proliferation of ACL and MCL fibroblasts in skeletally mature rabbits treated with PDGF-AB, EGF, and bFGF. The growth-factor treated fibroblasts proliferated significantly more than untreated fibroblasts. However, when compared with previous results on skeletally immature fibroblasts, the proliferation was found to decrease with skeletal maturation. This suggests that not only are the cell type and presence of growth factors important, the inherent response of the cell may differ depending upon the age of the patient.

The growth factor applications in ligament healing continue to be extensively investigated, though delivery methods remain a challenge. In 1999, Menetrey et al[39] demonstrated the feasibility of directly transferring transduced fibroblasts and myoblasts into the ACL of rabbits using the LacZ reporter gene. Gene-enhanced delivery methods may allow the successful introduction of bioactive factors in the future.

Meniscus Healing

Meniscus repair and preservation continues to be a goal of most orthopedic surgeons. Clinical outcomes after meniscal repair remain somewhat limited particularly as it relates to partial or incomplete healing in the face of a "clinically successful" repair. Cannon and Vittori[40] demonstrated meniscus repair in the presence of ACLR resulted in a 93% rate of healing compared with 50% healing in repairs performed in stable knees. Incomplete healing of meniscal repairs, however, has been well documented in studies reporting on second-look arthroscopy data. Tenuta and Arciero[41] documented a 20% incomplete healing rate at 11 months in knees undergoing inside-out suture repair and ACLR. Asahina et al[42] performed routine second-look arthroscopy on 86 knees undergoing inside-out suture repair of meniscal tears with concomitant ACLR, documenting a 15% rate of incomplete healing. These studies suggest that bioactive augmentation of meniscal repair site healing may play a role.

The basic science work of Arnozcky using autologous fibrin clot to augment isolated (non-ACLR) meniscus repairs has demonstrated the value of this

technique as a viable biologic method.[43] Clinical evidence after the use of fibrin clot has documented increased healing rates in isolated and avascular tears of the meniscus. Henning et al[44] demonstrated a 17% increase in healing rate with the use of fascial sheath and fibrin clot. The surgical technique for the use of a fibrin clot involves the use of 30 to 50 mL of venous blood drawn from the patient by the anesthesiologist and placed in a sterile glass container. A sintered glass rod is then used to stir the blood until a clot forms. The clot is then delivered to the repair site arthroscopically.

More recently, interest in fibrin clot techniques and the expanded applications of growth factors in meniscal healing has led to the development of platelet-rich fibrin matrix (PRFM). PRFM represents a potentially more precise technique to introduce a more concentrated and volume-stable fibrin matrix rich in platelets.[45] The PRFM technique also uses autologous venous blood (9 mL) which is transferred to a proprietary blood tube collection kit (Cascade Autologous Platelet System, MTF, Edison, NJ). The autologous blood is centrifuged for 15 minutes separating out the red blood cells from the platelet-rich plasma. (Figs. 2A, B) The platelet-rich plasma is then further spun down for 6 minutes to concentrate the plasma producing a volume-stable suturable fibrin matrix loaded with platelets. Arthroscopic insertion of the PRFM is performed at the time of meniscal repair and may be applicable to all of the potential repair methods including inside-out and outside-in suturing as well as in conjunction with the use of fixators and hybridized suture devices and systems. The suturing of the fibrin clot can be performed with one of the suture-based systems by inserting the clot through a 5 mm diameter cannula with the diaphragm removed. The clot is inserted on the tibial side of the repair. (Figs. 3A and B) This delivery method has clinical applications in the repair of rotator cuff tendons and combined ligament/soft tissue graft reconstruction (Fig. 4). At the present time, evidence-based clinical outcome studies have been initiated to validate the methodology in meniscus repair and rotator cuff repair.

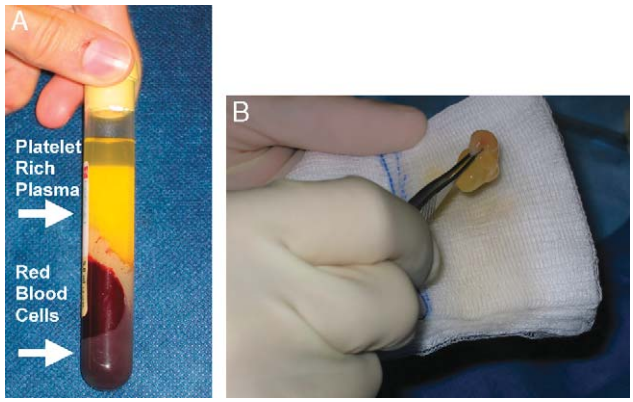

FIGURE 2. A, Image of autologous blood taken intraoperatively and spun down in a centrifuge. B, Image of PRFM obtained from the centrifuged plasma.

Articular Cartilage

The tendency of articular cartilage to respond to injury in a disordered and unpredictable manner contributes to the challenge of treating focal hyaline cartilage defects. Partial thickness injury to articular cartilage usually is associated with no appreciable healing whereas in a more significant osteochondral defect that penetrates the subchondral bone some element of fibrous or fibrocartilageneous healing may be realized. Marrow stimulation including microfracture, which has been increasingly accepted as a minimally invasive and practical primary approach to treating focal defects is predicated upon accessing the underlying subchondral bone and adjacent marrow rich in blood cells and pluripotent cells that may amplify repair of the overlying chondral defect. The potential for improving upon microfracture methods to optimize a repair response using bioactive factors is currently under investigation.

Erickson et al[46] studied rhBMP-2 using chondrocytes cultured from rat models and transfected with rhBMP-2 and found optimal cell proliferation, differentiation, and matrix synthesis. Sellers et al[47] used rhBMP-2 to repair osteochondral defects in the femurs of rabbits and found improved histologic results and subchondral healing at multiple time points with the rhBMP-2 samples.

Recent work found IGF-1 and TGF-β to be effective in articular cartilage restoration. In the presence of interleukin-1, a degradative inflammatory cytokine, IGF-1 and TGF-β restored proteoglycan synthesis to a

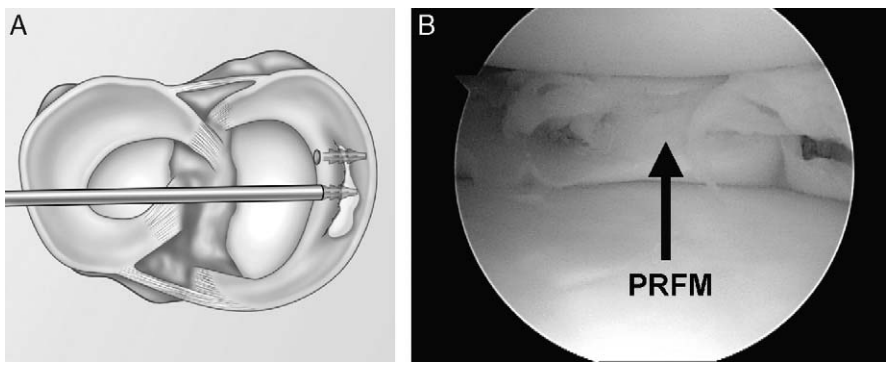

FIGURE 3. A, Illustration of PRFM insertion into the meniscus. B, Image of PRFM insertion into the meniscus.

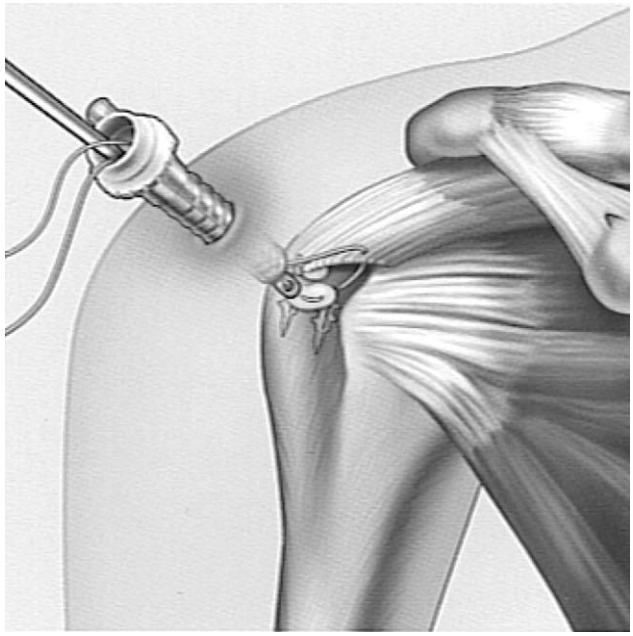

FIGURE 4. Illustration of PRFM insertion into the rotator cuff tendon.

control level, whereas BMP-2 improved expression to even greater levels.[48] Goldberg et al[49] reported on cultured chondrocytes used in an autologous chondrocyte implantation procedure and treated in a medium containing TGF-β and compared them to controls. The study found significantly greater amounts of DNA and glycosaminoglycans in the TGF-β enhanced medium than in controls.[49] Cook et al[50] studied the effect of OP-1 on 5 mm osteochondral defects in 65 adult dogs and found defects treated with OP-1 exhibited healing that histologically resembled mature hyaline cartilage compared with the fibrocartilage found in controls. Furthermore the repair sites were grossly continuous with adjacent cartilage compared with the irregularity found in controls.[50]

Basic science work in the area of articular cartilage defect resurfacing will continue to evolve. Many advances have already been made and although results obtained with current chondral restoration treatment techniques have been encouraging, the use of bioactive factors such as TGF-β, IGF-1, OP-1, and rhBMP-2 offers further therapeutic promise.

Limitations

Many studies have shown great benefits from use of individual and multiple growth factors; however, the dosing and transfer of these bioactive proteins to their desired targets remains a challenge. The ideal method of delivery should be easily and reliably delineated, cost effective, and clearly controlled. One effective means for delivery may include the use of a biodegradable and biocompatible scaffold, comprised of a polymer biomaterial that is volume-stable and can be surgically implanted into the repair site. The scaffold could then be impregnated ex vivo with growth factors that would be released at varying time points in the repair curve.

Another means to deliver growth factors is through gene-enhanced tissue engineering. In this model, a candidate gene which produces the beneficial growth factor protein may be transduced into the specific musculoskeletal target cells (ie, fibroblasts) which in turn activate the production of the particular therapeutic factors thereby enhancing repair. Both viral and nonviral vectors may be used to introduce the candidate gene into the target cell.[51] Transfection with nonviral vectors includes the use of naked DNA, liposomes, and biolistics (gene gun). The advantages of nonviral vectors are their low immunogenic potential and their ease of manufacturing. Although improvements have been made in the use of nonviral vectors, efficiency of gene transfer remains low. Transduction with viral vectors includes the use of adenovirus, adenoassociated virus, herpes simplex, Maloney murine leukemia, and lentivirus. The viral vectors typically are highly efficient, infect dividing and nondividing cells, and have a large capacity for transfer. However, they can be difficult to engineer and the ability of retroviruses to inadvertently activate promoter genes all along the host DNA remains a concern.

A limitation of gene-modified tissue engineering remains the inability to control the extent of gene activity after transfer. Currently, the ability to specifically target certain cell types or tissues although limiting the effect on adjacent tissues is unsatisfactory. Furthermore, a point is reached in the repair when the amount of growth factor begins to undergo catabolic rather than anabolic effects. Complications are of particular concern in that the therapeutic effects of certain bioactive factors may not always be effectively controlled. The use of angiogenic factors (VEGF) to promote neovascularization and new vessel ingrowth can be associated with an exuberant anabolic scar response with resultant arthrofibrosis. In addition aberrant phenotypic differentiation of chondrocytes or fibroblasts after application of improperly dosed growth factors can result in osteoblast expression and heterotopic ossification. Significant morbidities could be realized if neoplastic or benign tumorous cell behavior occurred. These issues regarding the precise control and dosing of exogenous factors remain of great importance. Furthermore, challenges remain with gene therapy including the preparation, packaging, and delivering the constructs as well as the cost of research and development of these products. Certainly, a cost-effective solution needs to be considered and preferably reached. Finally, regulatory issues remain a significant problem as it relates to the clinical application and accepted use of bioactive agents. Federal Drug Administration review and licensing approvals remain rigorous and thorough demonstration of both safety and efficacy is mandatory. These considerations clearly affect realistic timelines and widespread relevance.

Future Strategies

The future of bone and soft tissue repair will likely be based on biologic augmentation of healing and tissue regeneration. Clinicians continue to anticipate the potential application of innovative growth factors and new delivery techniques. The clinical value and efficacy of new designs will be realized to supplement and augment strong mechanical repairs. The use of gene activated matrix is a method of nonviral delivery of genes to skeletal defects for repair. It is a plasmid DNA that encodes for a gene that amplifies growth factor production and can be loaded onto a collagen scaffold and implanted into a bone defect. Migrating cells become transiently transduced to express a specific gene and enhance repair. This has shown some potential in the healing of bone defects when compared with controls.[52] The benefits include regulated and localized delivery of therapeutic genes and the ability to achieve a gene-modified intervention using a nonviral method.

Practical delivery methods are being developed that use autologous sources obtained at the index point of intervention and are available to the surgeon in the operating room. One such method includes the intraoperative concentration of autologous mesenchymal stem cells from bone marrow harvested intraoperatively. The marrow is obtained via a large bore needle, which is then transferred into the commercially designed system (Cellect, Depuy Spine, Warsaw, IN) for concentration. This technique is currently being clinically studied in spinal fusion.

Summary

The use of biologically active factors currently holds great promise in the field of sports medicine and arthroscopy. Numerous potential applications exist for the augmentation and manipulation of soft tissue and bony healing and repair. Basic science advances may ultimately be clinically introduced, yet more work remains in the area of precise growth factor characterization, control, dosing, and delivery. Most importantly evidenced-based controlled comparison data must be collected and studied to validate the use of these promising technologies and justify their cost-effectiveness. In the near future, orthopedics will likely depend more upon the use of biologic and biochemical methods in combination with established fixation and mechanical methods.

REFERENCES

1. Musgrave DS, Fu FH, Huard J. Gene therapy and tissue engineering in orthopaedic surgery. *J AAOS*. 2002;10:6–15.
2. Huard J, Fu FH. *Gene Therapy and Tissue Engineering in Orthopaedic and Sports Medicine*. Boston MA: Birkhäuser; 2000:196–212.
3. Newman A. Current concepts: articular cartilage repair. *Am J Sports Med*. 1998;26:309–324.
4. Akeson WH, Bugbee W, Chu C, et al. Differences in mesenchymal tissue repair. *Clin Ortho Rel Res*. 2001;391:S124–S141.
5. Davidson JM, Zola O, Liu JM. Modulation of transforming growth factor-beta stimulated elastin and collagen production and proliferation in porcine vascular smooth cells and skin fibroblasts by basic fibroblast growth factor, transforming growth factor-alpha, and insulin-like growth factor-1. *Cell Physiol*. 1993;155:149–156.
6. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. *Tissue Eng*. 1999;1: 345–353.
7. Smith C, Storms B. Hematopoietic stem cells. *Clin Orthop*. 2000; 379:S91–S97.
8. Lieberman J, Daluiski A, Einhorn T. Current concepts review: the review of growth factors in the repair of bone. *J Bone Joint Surg*. 2002;84:1032–1044.
9. Boden S. Bioactive factors for bone tissue engineering. *Clin Orthop Rel Res*. 1999;367S:S84–S94.
10. Wang EA, Rosen V, D'Alessandro JS, et al. Recombinant human bone morphogenetic protein induces bone formation. *Proc Natl Acad Sci U S A*. 1990;87:2220–2224.
11. Zegzula HD, Buck DC, Brekke J, et al. Bone formation with the use of rhBMP-2 recombinant bone morphogenetic protein-2. *J Bone Joint Surg Am*. 1997;79:1778–1790.
12. Khouri RK, Brown DM, Koudsi B, et al. Repair of calvarial defects with flap tissue: role of bone morphogenetic proteins and competent responding tissues. *Plast Reconstr Surg*. 1996;98: 103–109.
13. Friedlaender GE, Perry CR, Cole JD, et al. Osteogenic protein-1 (bone morphogenic protein-7) in the treatment of tibial nonunions. *J Bone Joint Surg Am*. 2001;83:S151–S158.
14. Cook SD, Wolfe MW, Salkeld SL, et al. Effect of recombinant human osteogenic protein-1 on healing of segmental defects in non-human primates. *J Bone Joint Surg Am*. 1995;77:734–750.
15. Lee JY, Musgrave D, Pelinkovic D, et al. Effect of bone morphogenic protein-2-expressing muscle-derived cells on healing of critical sized bone defects in mice. *J Bone Joint Surg Am*. 2001; 83:1032–1039.
16. Govender S, Csimma C, Genant HK, et al. BMP-2 evaluation in surgery for tibial trauma (BESTT) study group. Recombinant human bone morphogenic protein-2 for treatment of open tibial fractures: a prospective controlled, randomized, study of four hundred and fifty patients. *J Bone Joint Surg Am*. 2002;84: 2123–2134.
17. Berkus JK, Transfeldt E, Kitchel S, et al. Clinical and radiographic outcomes of anterior lumbar interbody fusion using recombinant human bone morphogenic protein-2. *Spine*. 2002;27:2396–2408.
18. Boden SD, Titus L, Hair G, et al. Lumbar spine fusion by local gene therapy with a cDNA encoding a novel osteoinductive protein (LMP-1). *Spine*. 1998;23:2486–2492.
19. Minamide A, Boden S, Viggeswarpu M. Mechanism of bone formation with gene transfer of the cdna encoding for the intracellular protein lim mineralization protein-1. *J Bone Joint Surg Am*. 2003;85:1030–1039.
20. Ranly DM, McMillan J, Keller T, et al. Platelet-derived growth factor inhibits demineralized bone matrix-induced intramuscular cartilage and bone formation. *J Bone Joint Surg Am*. 2005; 87:2052–2064.
21. Scherping SC, Schmidt CC, Georgescu HI, et al. Effect of growth factors on the proliferation of ligament fibroblasts from skeletally mature rabbits. *Connect Tissue Res*. 1997;36:1–8.
22. Rodeo S, Arnoczky SP, Torzilli P, et al. Tendon healing in a bone tunnel, a biomechanical and histologic study in the dog. *J Bone Joint Surg Am*. 1993;75:1795–1803.
23. Schiavone PA, Milano G, Luciania L, et al. Graft healing after anterior cruciate ligament reconstruction in rabbits. *Clin Orthop*. 1997;343:203–212.
24. Park MJ, Lee MC, Seong SC. A comparative study of the healing of tendon autograft and tendon-bone autograft using patellar tendon in rabbits. *Int Orthop*. 2001;25:35–39.
25. Rodeo S, Suzuki K, Deng X, et al. Use of recombinant bone morphogenic protein-2 to enhance tendon healing in a bone tunnel. *Am J Sports Med*. 1999;27:476–488.
26. Anderson K, Aruna S, Kazataka I. Augmentation of tendon healing in an intraarticular bone tunnel with use of a bone growth factor. *Am J Sports Med*. 2001;29:689–698.

27. Martinek V, Latterman C, Usas A. Enhancement of tendon-bone integration of anterior cruciate ligament grafts with bone morphogenic protein-2 gene transfer. *J Bone Joint Surg Am*. 2002;84: 1123–1131.
28. Mihelic R, Pecina M, Jelic M, et al. Bone morphogenic protein-7 (osteogenic protein-1) promotes tendon graft integration in anterior cruciate ligament reconstruction in sheep. *Am J Sports Med*. 2004; 32:1619–1625.
29. Clark RT, Johnson TL, Schalet BJ, et al. GDF-5 deficiency in mice leads to disruption of tail tendon form and function. *Connective Tissue Res*. 2001;42:175–186.
30. Chabra A, Tsou D, Clark R. GDF-5 deficiency in mice delays achilles tendon healing. *J Ortho Res*. 2003;21:826–835.
31. Lou J, Tu Y, Ludwig F, et al. Effect of bone morphogenetic protein-12 gene transfer on mesenchymal progenitor cells. *Clin Orthop Rel Res*. 1999;369:333–339.
32. Lou J, Tu Y, Burns M. Bmp-12 gene transfer augmentation of lacerated tendon repair. *J Orthop Res*. 2001;19:1199–1202.
33. Lee J, Chamberlin TA, Schreck PJ, et al. In situ localization of growth factors during the early healing of knee ligaments. *Trans Orthop Res Soc*. 1995;20:158.
34. Sciore P, Boykiw R, Hart DA. Semiquantitative reverse transcriptase polymerase chain reaction analysis of mRNA for growth factors and growth factor receptors from normal and healing rabbit medial collateral ligament tissue. *J Orthop Res*. 1998;16:429–437.
35. Marui T, Niyibizi C, Gworgescu HI, et al. The effect of growth factors on matrix synthesis by ligament fibroblasts. *J Orthop Res*. 1997;15:18–23.
36. DesRosiers EA, Yahia L, Rivard C-H. Proliferative and matrix synthesis response of canine anterior cruciate ligament fibroblasts submitted to combined growth factors. *J Orthop Res*. 1996; 14:200–208.
37. Batten ML, Hansen JC, Dahners LE. Influence of dosage and timing of application of platelet derived growth factor on early healing of the rat medial collateral ligament. *J Orthop Res*. 1996;14:736–741.
38. Evans CH. Cytokines and the role they play in the healing of ligaments and tendons. *Sports Med*. 1999;28:72–76.
39. Menetrey J, Kasemkijurattana C, Day C. Direct fibroblast and myoblast mediated gene transfer to the anterior cruciate ligament. *Tissue Eng*. 1999;5:435–442.
40. Cannon W, Vittori J. The incidence of healing in an arthroscopic meniscal repairs in anterior cruciate ligament-reconstructed knees versus stable knees. *Am J Sports Med*. 1992;20:176–181.
41. Tenuta JJ, Arciero R. Arthroscopic evaluation of meniscal repairs. *Am J Sports Med*. 1994;22:797–802.
42. Asahina S, Mureta T, Hoshinu A. Intermediate results of meniscal repair in anterior cruciate ligament reconstruction knees. *Am J Sports Med*. 1998;26:688–691.
43. Arnoczky SP, Warren RF, Spivak JM. Meniscal repair using an exogenous fibrin clot. An experimental study in dogs. *J Bone Joint Surg Am*. 1988;70:1209–1217.
44. Henning CE, Lynch MA, Yearout KM. Arthroscopic meniscal repair using exogenous fibrin clot. *Clin Ortho*. 1990;252:64–72.
45. Sgaglione NA. Meniscus repair update: current concepts and new techniques. *Orthopedics*. 2005;28:280–286.
46. Erickson DM, Harris SE, Dean DD, et al. Recombinant bone morphogenetic protein (BMP-2) regulates costochondral growth plate chonrocytes and induces expression of BMP-2 and BMP-4 in a cell maturation-dependent manner. *J Orthop Res*. 1997;15: 371–380.
47. Sellers RS, Peluso D, Morris EA. The effect of recombinant human bone morphogenetic protein-2 (rhBMP-2)on the healing of full-thickness defects of articular cartilage. *J Bone Joint Surg Am*. 1997;79:1452–1463.
48. Smith P, Shuler FD, Georgescu HI, et al. Genetic enhancement of matrix synthesis by articular chondrocytes: comparison of different growth factor genes in the presence and absence of interleukin-1. *Arthritis Rheum*. 2000;43:1156–1164.
49. Goldberg AJ, Lee DA, Bader DL. Autologous chondrocytes implantation. Culture in a tgf-b containing medium enhances the expression of a chondrocytes phenotype in passaged human chondrocytes in pellet culture. *J Bone Joint Surg Br*. 2005;87: 128–134.
50. Cook S, Patron L, Salkeld S, et al. Repair of articular cartilage defects with osteogenic protein-1 in dogs. *J Bone Joint Surg Am*. 2003;85:116–123.
51. Hannallah D, Peterson B, Lieberman J. Gene therapy in orthopaedic surgery. *J Bone Joint Surg Am*. 2002;84:1046–1061.
52. Bonadio J, Smiley E, Patil P, et al. Localized, direct plasmid gene delivery in vivo: prolonged therapy results in reproducible tissue regeneration. *Nat Med*. 1999;5:753–759.

