

Biologic Treatments for Sports Injuries II Think Tank—Current Concepts, Future Research, and Barriers to Advancement, Part 1

Biologics Overview, Ligament Injury, Tendinopathy

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Biologic therapies, including stem cells, platelet-rich plasma, growth factors, and other biologically active adjuncts, have recently received increased attention in the basic science and clinical literature. At the 2015 AOSSM Biologics II Think Tank held in Colorado Springs, Colorado, a group of orthopaedic surgeons, basic scientists, veterinarians, and other investigators gathered to review the state of the science for biologics and barriers to implementation of biologics for the treatment of sports medicine injuries. This series of current concepts reviews reports the summary of the scientific presentations, roundtable discussions, and recommendations from this think tank.

Keywords: mesenchymal stem cells; platelet-rich plasma; growth factors; ligament injury; tendinopathy; rotator cuff; cartilage

There has long been an interest in biologics for treatment of sports medicine injuries, although the past few decades of research have largely focused on anatomic, biomechanical, and clinical outcome studies of surgical treatments for ligament, tendon, rotator cuff, and cartilage injuries.^{51,55,69,71} Biologic therapies may augment healing by improving the biomechanical quality of healing tissue and helping to restore native tissue. However, there are still many critical gaps in understanding the basic science, translational use, and optimal clinical applications of biologics.

The incorporation of biologics into routine clinical practice may result in a shift in the care of sports injuries,

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similar to that observed when sports medicine adopted the use of the arthroscope, advancing the care of both athletes and the general population. This will require the development of analytic tools with a high sensitivity, specificity, and selectivity to assess healing, tissue quality, and clinical outcomes.⁵⁰ Patient-based outcomes data are critical to prove safety and efficacy and will be essential in acquiring US Food and Drug Administration (FDA) approval, establishing procedural reimbursement codes, and facilitating widespread use in clinical care.

The purpose of this current concepts review is to present the findings of the 2015 AOSSM Biologics II Think Tank, synthesizing the current state of the literature and future direction of both laboratory and clinical studies on the use of biologics for treatment of sports medicine injuries. Part 1 of this series includes an overview of mesenchymal stem cells (MSCs), growth factors and cytokines, and platelet-rich plasma as well as the regulatory environment. The use of biologic therapies in the treatment of ligament injuries and tendinopathy is also reviewed. Parts 2 and 3 (published in the *Orthopaedic Journal of Sports Medicine*) focus on the use of biologics in the treatment of rotator cuff and articular cartilage pathology, respectively.

CURRENT STATUS OF STEM CELLS IN REGENERATIVE APPLICATIONS IN SPORTS MEDICINE

MSCs have the potential to contribute to tissue regeneration directly by differentiation into damaged cell types or

TABLE 1
The Use of Stem Cells in Orthopaedic Surgery: Targeted Areas for Future Research
and Barriers to Clinical Implementation^a

Targeted areas

- Characterization of the appropriate tissue environment into which certain MSCs are transplanted
- Clarification of the mechanism of MSC therapy—paracrine effect, immunomodulation, or direct engraftment
- Identification of factors that stimulate release, recruitment, and activation of native stem cells
- Development of serum-free media for MSC culture and expansion
- Identification of novel genetic markers and subsets of MSCs (including pericytes) that specialize in different tissue types
- Development of methods to obtain purified autograft perivascular MSCs
- Recruitment of resident (tissue-specific) stem cells
- Robust large animal and clinical trials

Barriers

- Limited availability of MSCs in host tissues and probable need for expansion before injection
 - Complexity and cost of regulatory system
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^aMSC, mesenchymal stem cell.

indirectly by stimulating angiogenesis, limiting inflammation, and recruiting local tissue-specific progenitors.⁷ MSCs are adult stem cells and are believed to be present within almost every tissue in the body.⁷⁰ Minimum criteria to define MSCs were provided in a consensus statement by the International Society for Cellular Therapy (ISCT).³¹ The ISCT criteria stated that cells must be plastic adherent, express certain cell surface antigens (CD105, CD73, and CD90) but not others (CD45, CD34, CD14, CD11b, CD79 α , CD19, or human leukocyte antigen–antigen D related), and have the capacity to differentiate into osteoblasts, adipocytes, and chondroblasts in vitro. The following is an overview of MSCs for use in orthopaedic surgery, and Table 1 provides a summary of targeted areas for future research and barriers to clinical implementation.

MSC Sources and Purification

A range of MSC preparations are available, which vary in tissue source, whether the MSC populations within preparations have been enriched through culture or machine purified, and typical yield. Table 2 summarizes the major groups of MSC preparations being studied in the field of orthopaedics. Irrespective of tissue source, and by definition, MSCs can be driven to a chondrogenic, osteogenic, or adipogenic fate among other lineages. This is routinely achieved in laboratory culture by supplementation with lineage-specific growth factor combinations. For example dexamethasone, β -glycophosphate, and ascorbic acid are used to promote osteogenic differentiation.⁷⁴ Whether the desired lineage is induced before MSC delivery is an area of ongoing research.⁸⁵

MSCs were first isolated from bone marrow, which remains the most common clinical source because of its accessibility to surgeons and the extensive laboratory characterization of bone marrow–derived MSCs.⁷² Small-volume bone marrow aspirates (usually less than 4–5 mL) are preferred for obtaining MSCs because further volume extraction results in hemodilution, likely owing to mixing with peripheral blood.² Although MSCs make up a small minority of cells within bone marrow (less than 1/10,000 cells), unpurified

preparations (eg, concentrated bone marrow aspirate) have been used directly with the aim of harnessing the potential of contained MSCs.¹⁵ However, available studies demonstrate that these heterogeneous populations, including inflammatory cells, hematopoietic cells, endothelial cells, and nonviable cells, may result in poor and inconsistent tissue formation compared with enriched MSC preparations.

Currently, clinical-grade bone marrow–derived or adipose-derived MSCs are grown and expanded in serum-based media; the use of serum-free media with necessary growth factors to minimize both potential immunologic responses and the risk of contamination remains an area needing further investigation. However, there is evidence that long-term culture is associated with genetic instability and a reduction in therapeutic potency.⁸⁷ Production of clinically utilized MSCs requires facilities that comply with good manufacturing practices. However, cell expansion in culture is considered “manipulation,” which currently renders this technique as not viable for clinical practice in the United States.⁷

Adipose tissue is the other main clinical source of MSCs, referred to as adipose-derived stem cells. They have a higher yield than bone marrow–derived MSCs and are harvested from adipose aspirates or liposuction.^{9,103} The infrapatellar fat pad has also been identified as a source for adipose-derived stem cells.³³

Methods for separation of cells have been designed and are commercially available, including several centrifugation systems and other mechanical systems. Rapisio et al⁸³ described a system that utilized vibration as a means to separate cells. Ultrasound-based devices have also been described, although there is concern for cell death due to thermal energy, which may be addressed using pulsed systems. A filtration-based system has also been described for cell isolation.¹⁴

Perivascular MSCs (Pericytes and Adventitial Cells)

It was recently demonstrated that 2 populations of perivascular cells can adopt an MSC-like phenotype.⁷³ Microvascular pericytes and adventitial cells that reside within the tunica adventitia of larger vessels fulfil all aspects of ISCT criteria

TABLE 2
Mesenchymal Stem Cell Preparations for Orthopaedic Applications^a

Preparation	Tissue Source	Harvest/Preparation Technique	Preparation Time (Approximate)	Typical Yield	Purity	Likely FDA Pathway
Concentrated bone marrow aspirate	Marrow	1. Bone marrow aspirate 2. Centrifugation 3. Delivery	30 min	1×10^4 /mL bone marrow aspirate ²²	Heterogeneous	N/A
SVF	Adipose	1. Lipoaspirate 2. Digestion 3. Centrifugation 4. Delivery	90 min	31×10^6 per 200 mL lipoaspirate ⁴⁵	Heterogeneous	351/361
BM-MSC	Marrow	1. Bone marrow aspirate 2. Centrifugation 3. Cell culture 4. Delivery	2-4 wk	Unlimited through culture expansion	Culture enriched	351
ADSC	Adipose	1. Lipoaspirate 2. Digestion 3. Centrifugation 4. Cell culture 5. Delivery	2-4 wk	Unlimited through culture expansion	Culture enriched	351
PSC	Marrow, adipose, or vascularized tissue	1. Tissue harvest 2. Digestion 3. Centrifugation 4. FACS purification 5. Delivery	180 min	31×10^6 per 200 mL lipoaspirate ⁴⁵	Purified	351

^aADSC, adipose-derived mesenchymal stem cell; BM-MSC, bone marrow–derived mesenchymal stem cell; FACS, fluorescence-activated cell sorting; FDA, US Food and Drug Administration; MSC, mesenchymal stem cell; N/A, not applicable; PSC, perivascular stem cell or perivascular mesenchymal stem cell; SVF, stromal vascular fraction.

defining MSCs and can be purified to homogeneity using fluorescence-activated cell sorting (FACS).^{26,27} Unlike conventionally derived MSCs (adipose-derived stem cells or bone marrow–derived MSCs), the processes used to isolate perivascular MSCs do not require extended periods of laboratory culture.⁷⁴ It is not yet clear whether all MSC populations, including those isolated from laboratory culture, are actually derived from perivascular cells.¹⁸ A major theorized advantage of isolating perivascular MSCs using FACS is the high yields that can be purified and delivered immediately without any of the delays and risks associated with laboratory culture.⁷² Up to 31 million MSCs may be yielded from just 200 mL of lipoaspirate.⁴⁵

Tissue-Specific Stem Cells

In addition to use of bone- and adipose-derived stem cells, some investigators have evaluated the characteristics of tissue-specific stem cells. Matsumoto et al⁶⁰ studied human anterior cruciate ligament (ACL)–derived vascular stem cells; they identified that the ACL septum region contains a population of stem cells and theorized that these cells may play a role in healing. Randelli et al⁸² harvested samples of rotator cuff and proximal biceps tendons during rotator cuff surgery; resident cells were identified to have adult stem cell characteristics, were cultured in vivo, and were able to undergo differentiation into different cell types. However, it has been reported that diseased rotator cuff tissues

have a lower number of resident MSCs, which may limit the potential for in situ activation or ex vivo cell culture.⁴³

Clinical Use of MSCs

The clinical use of MSCs and associated outcomes of treatment are being investigated and are reviewed in the subsequent sections on ligament injury, tendinopathy, rotator cuff tears, and articular cartilage defects. However, there remain several unanswered questions regarding the clinical use of MSCs, and defining the specific growth factors, local cellular interactions, and the local survival and degree of differentiation of MSCs will be required to allow observations of clinically or objectively measurable improvements.

GROWTH FACTORS AND CYTOKINES FOR REGENERATIVE APPLICATIONS IN SPORTS MEDICINE

Growth factors are one of the key regulators of the normal response to injury, tissue regeneration, and healing. Harnessing the capacity of growth factors to promote cellular proliferation, migration, survival, and differentiation while contributing to angiogenesis may form an integral part of future therapies in orthopaedic sports medicine. The majority of growth factors are pleiotropic, causing multiple biological effects, with some stimulating changes in

numerous cell types. Growth factors tend to exist in families of structurally related proteins binding with large, specific transmembrane receptor molecules present on the surface of target cells. As such, the presence or absence of specific receptors defines a cell's ability to respond to any given factor.

Growth factors can be delivered individually or as synergistic combinations directly to sites of injury, where they act directly on host cells to bring about their therapeutic effect. In addition, growth factors are increasingly being used in combination with MSCs, whose ability to differentiate into bone, fat, muscle, and cartilage while beneficially modifying local immune environments and creating a regenerative microenvironment has made them a promising substrate for musculoskeletal regeneration.¹⁹ Concomitant delivery of growth factors may augment the regenerative potential of transplanted MSCs while optimizing a regenerative microenvironment through actions on cells within target tissues. In addition, growth factors are playing an increasing role in the preparation and preconditioning of MSCs in laboratory culture before delivery. There is currently great interest from basic scientists and translational researchers in the use of growth factor-supplemented (serum-free) media for MSC culture to end the reliance on animal products such as fetal bovine serum, which have a theoretical risk of immune reactions and infection.^{52,86}

Clinical Considerations and Future Challenges

A large number of growth factors and cytokines have effects relevant to the regeneration of musculoskeletal tissue and therefore represent potential therapeutic targets (Table 3). A number are already being used to the benefit of orthopaedic patients, with bone morphogenetic protein (BMP) 2 and BMP7 demonstrating a beneficial effect on fracture healing in randomized controlled trials.^{36,40} Some agents such as fibroblast growth factor have shown promise in clinical studies,⁴ whereas others, including transforming growth factor (TGF)- β , have been evaluated in small animals.^{42,78} Combination treatments, including the use of platelet-rich plasma (PRP), are drawing much attention because of the synergistic effects of many growth factors. However, the diverse actions of growth factors, often varying with dose, cell type, and host factors, highlights the importance of tailoring any potential growth factor-based treatments to an individual patient's injury. Each clinical situation represents a unique microenvironment with different numbers of host progenitors, variations in levels of endogenous growth factors, and variable receptor expression. Furthermore, the type of cells present and the number of growth factor receptors on these cells are also known to vary at different stages of the healing process. This must be considered when extrapolating experimental evidence and clinical studies into clinical practice. More studies are required to evaluate the range of agents and combinations available and to determine the optimum methods, dosing, and time of delivery of growth factors in sports medicine.

A challenge for future growth factor-based therapies will be to confirm the long-term safety of growth factor treatments in addition to their efficacy. The vast majority

of growth factors being investigated for orthopaedic applications have multiple biologic actions beyond the musculoskeletal system. The potential for harmful off-target effects must therefore be fully evaluated at each stage of therapy development. Failure of this process was highlighted by the catastrophic complications reported after BMP2 delivery for spinal fusion.²⁰

Concerns have also been raised regarding the genetic stability of MSCs treated with growth factors in culture,¹² particularly given the immunosuppressive effects of MSCs and the theoretical risk for malignant transformation. In this regard, particular consideration must be paid to growth factor delivery, which should ideally be localized and of a time-limited nature. Controlled release of growth factors or presentation of the growth factor in bioengineered form are some of the ways in which this may be achieved.

CURRENT STATUS OF PRP IN REGENERATIVE APPLICATIONS IN SPORTS MEDICINE

Autologous PRP has become increasingly utilized in clinical applications as a theoretical adjunct to musculoskeletal tissue healing because of the presence of several growth factors that may promote healing. PRP is defined as a sample of autologous blood with platelet concentrations above baseline produced by the centrifugal separation of whole blood.⁵⁹ In addition to platelets, PRP contains varying levels of leukocytes (namely, monocytes and neutrophils) that may either positively or negatively affect the repair process. The concentration of platelets and leukocytes in individual PRP preparations may be variable depending on the system utilized²¹ and there are significant variations reported even within an individual patient over a 2-week time period.⁶¹ An overview of PRP contents (Table 4), preparations, and basic science is provided. In addition, Table 5 includes a summary of targeted areas for future research and barriers to clinical implementation.

PRP Contents

PRP contains platelets, plasma, leukocytes, and erythrocytes (although in small numbers). To date, more than 300 distinct molecules have been detected in platelet releasates.²⁵ The major components of PRP and their selected contents/releasates relevant to orthopaedic regeneration are summarized in Table 4. PRP contains several important growth factors that can enhance tissue healing by serving as chemoattractants and stimulators of cell proliferation, such as TGF β , platelet-derived growth factor, insulin-like growth factor, and vascular endothelial growth factor (VEGF). Once activated, near-complete release of growth factors from platelets occurs within 1 hour and the half-life is on the order of minutes to hours. This underscores the importance of appropriate timing of PRP application and may support a series of injections.

PRP also contains varying concentrations of leukocytes depending on the method of preparation. Leukocyte concentration in PRP may be compared with the concentration in whole blood and categorized as leukocyte rich (LR) or

TABLE 3
Effects Relevant to Musculoskeletal Regeneration of Selected Cytokines and Growth Factors^a

Growth Factor	Effects Relevant to Orthopaedic Regeneration	Supporting Studies ^b
BMP2	MSC proliferation; osteogenic differentiation; chondrogenic differentiation; stimulates collagen production	1-4
BMP4	Osteogenic differentiation	2, 5
BMP6	Osteogenic differentiation	2, 5
BMP7	Osteogenic differentiation; stimulates collagen production	2, 5, 6
CTGF	Angiogenesis; cartilage regeneration; platelet adhesion	7, 8
EGF	Endothelial chemotaxis and angiogenesis; MSC and epithelial cell mitogenesis; collagen synthesis; modulates osteogenic and chondrogenic differentiation of MSCs	9, 10
FGF1	Stimulates proliferation of capillary endothelial cells	11-13
FGF2	Stimulates proliferation of capillary endothelial cells; MSC, chondrocyte, and osteoblast mitogenesis; chondrocyte, myoblast, and osteoblast differentiation; myogenic differentiation	11, 12, 14
FGF6		15
FGF7	Keratinocyte proliferation, migration, and differentiation	16-18
FGF10		16
HGF	Angiogenesis, mitogen for endothelial cells; antifibrotic; limits inflammatory response	19
IGF1	Myoblast proliferation and differentiation; chemotactic for fibroblasts and stimulates protein synthesis; enhances bone formation by proliferation and differentiation of osteoblasts; promotes MSC proliferation and survival; modulates chondrogenesis	20, 21
IL1	Proinflammatory, catabolic	22
PDAF	Increases vascularization by stimulating vascular endothelial cells	23
PDEGF	Promotes wound healing by stimulating proliferation of keratinocytes and dermal fibroblasts	24
PDGF	Chemotactic agent for inflammatory cells; angiogenesis; fibroblast chemotaxis and proliferation; enhances matrix synthesis (including collagen); MSC and osteoblast mitogenesis	13, 25, 26
PF4	Chemoattractant for neutrophils and fibroblasts; antiheparin agent	27, 28
MMPs	ECM remodeling (tissue degradation)	29
NELL1	Stimulates bone formation	30, 31
SDF1 α	Neutrophil chemotaxis; MSC chemotaxis; mediates suppressive effect of MSCs on osteoclastogenesis	32
TGF β 1	Activation and proliferation of fibroblasts; collagen II, proteoglycan, and ECM synthesis; upregulation of TIMP; endothelial chemotaxis and angiogenesis; inhibits proliferation of macrophages and lymphocytes; MSC proliferation; chondrogenic differentiation; osteogenic differentiation	13, 33-36
TGF β 2	MSC proliferation; chondrogenic and osteogenic differentiation	33-35
TGF β 3	MSC proliferation; chondrogenic and osteogenic differentiation	33-35
TIMPs	ECM remodeling (inhibit MMPs)	37, 38
VEGF	Stimulate angiogenesis and vasculogenesis; chemotactic for macrophages and granulocytes; vasodilatory (indirectly by release of nitrous oxide)	39
Wnt3a	Modulates MSC proliferation, MSC survival; modulates osteogenic and chondrogenic differentiation of MSCs	13, 40-44
Wnt5a	Modulates osteogenic and chondrogenic differentiation of MSCs	13, 43, 44

^aBMP, bone morphogenetic protein; CTGF, connective tissue growth factor; ECM, extracellular matrix; EGF, endothelial growth factor; FGF, fibroblast growth factor; HGF, hepatocyte growth factor; IGF, insulin-like growth factor; IL, interleukin; MMP, matrix metalloproteinase; MSC, mesenchymal stem cell; NELL, NEL-like protein; PDAF, platelet-derived angiogenesis factor; PDEGF, platelet-derived endothelial growth factor; PDGF, platelet-derived growth factor; PF, platelet factor; SDF, stromal cell-derived factor; TGF, transforming growth factor; TIMP, tissue inhibitor of metalloproteinases; VEGF, vascular endothelial growth factor.

^bReferences for supporting studies are found in the Appendix, available online at <http://ajsm.sagepub.com/supplemental>.

leukocyte poor (LP).⁸⁴ In general, preparations with higher concentrations of platelets also include more extraneous cells. As such, the systems with the highest concentrations of platelets tend to be LR.⁷⁷ Leukocytes have been associated with increased interleukin-1 and tumor necrosis factor- α , both of which are inflammatory cytokines as outlined in Table 4. Further clarification of the role of leukocytes in PRP and selection of LP versus LR PRP for certain clinical conditions is needed. In addition to platelet concentration, studies must also control for inclusion/exclusion of leukocytes to allow for comparison.

Although there are many important growth factors in PRP, it may also contain inflammatory cytokines and matrix metalloproteinases (MMPs) that can increase tissue damage. It has also been reported that PRP contains growth factors that may be beneficial for healing for one tissue and may be deleterious for another. For example, TGF β 1 has been reported to be beneficial for healing of tendon and ligament injuries,¹³ whereas it has been shown to be deleterious to muscle due to fibrosis⁴⁴ and may negatively affect articular cartilage. VEGF has been noted to promote angiogenesis and thereby tissue healing; however,

TABLE 4
Major Components of PRP and Selected Contents/Releasate^a

Component	Contents/Releasate
Platelets	
Alpha granules	Growth factors (eg, PDEGF, PDGF, TGF β 1, IGF1, bFGF, PDAF, PF4, EGF, VEGF, CTGF, HGF, SDF1 α), hemostatic factors (eg, Factor V, vWF, fibrinogen), angiogenic factors (eg, angiogenin, VEGF), antiangiogenic factors (eg, angiostatin, PF4), proteases (eg, MMP2, MMP9), necrotic factors (eg, TNF α , TNF β), and other cytokines
Dense granules/bodies	ADP, calcium, serotonin
Lysosomes	Lysosomal enzymes
Plasma	Proteins (eg, albumin, fibrinogen, globulins, complement, clotting factors), electrolytes (eg, sodium, chloride, potassium, calcium), hormones (eg, estrogens, progesterone, androgens, IGF1, ACTH, HGH), biomarkers (eg, osteocalcin, CD11b, protein C)
Leukocytes	
Neutrophils	Cytokines (eg, IL4, IL8, TNF α), proteases, bactericidal molecules, lysozymes
Eosinophils	Cytokines and growth factors (eg, VEGF, PDGF, TGF α , TGF β , ILs), plasminogen
Basophils	Histamine, proteases, heparin, leukotrienes
Monocytes	Cytokines and growth factors (eg, IL1, IL6, FGF, EGF, PDGF, VEGF, TGF β)
Erythrocytes (minimal numbers)	ATP, nitric oxide, hemoglobin, and free radicals

^aACTH, adrenocorticotropic hormone; ADP, adenosine diphosphate; ATP, adenosine triphosphate; bFGF, basic fibroblastic growth factor; CTGF, connective tissue growth factor; EGF, endothelial growth factor; HGF, hepatocyte growth factor; HGH, human growth hormone; IGF, insulin-like growth factor; IL, interleukin; MMP, matrix metalloproteinase; PDAF, platelet-derived angiogenesis factor; PDEGF, platelet-derived endothelial growth factor; PDGF, platelet-derived growth factor; PF, platelet factor; SDF, stromal cell-derived factor; TGF, transforming growth factor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor; vWF, von Willebrand factor.

TABLE 5

The Use of PRP in Orthopaedic Surgery: Targeted Areas for Future Research and Barriers to Clinical Implementation

Targeted areas

- Development of methods of preparation to allow tissue- and injury-specific customization by the removal of deleterious growth factors
- Standardization of reporting of PRP contents in laboratory and clinical studies is strongly recommended
- Determination of optimal PRP characteristics (eg, growth factors, leukocytes, platelet concentration) to augment healing of all tissue types
- Clarification of appropriate timing, dosing, and frequency of application for key tissue types, injuries, and surgical interventions

Barriers

- Variability of PRP contents depending on commercial system and patient characteristics
- Inconsistency of characteristics of PRP used in published clinical studies limits ability to make conclusions on appropriate use
- Limited adoption of existing classification systems for PRP

it has been found to negatively affect articular cartilage healing. Further research is recommended to categorize the growth factors present in PRP and determine methods of preparation to allow customization of PRP to be tissue specific by the removal of deleterious growth factors.

Preclinical Studies on PRP to Guide Clinical Use

Platelet concentration and the timing of application are important variables in the clinical use of PRP. In a laboratory study by Giusti et al,³⁸ it was found that a concentration of 1.5×10^6 platelets/ μ L in platelet gel was most effective for promotion of angiogenesis; a lower concentration resulted in reduced proliferation, whereas a higher concentration was inhibitory. A study by Yoshida et al⁹⁹ showed that a platelet concentration essentially equivalent to the concentration in whole blood was the best stimulator of ACL cell metabolism and procollagen gene expression, and it outperformed PRP

with a $5\times$ concentration by increasing cell metabolism, decreasing cell apoptosis, and increasing collagen gene expression. Weibrich et al⁹⁶ evaluated the effect of platelet concentration in PRP on peri-implant bone regeneration and found that an intermediate concentration (2-6 times the concentration of whole blood) was optimal, whereas higher concentrations were associated with an inhibitory effect. The downregulation of desired effects may be concentration dependent as a negative feedback loop or may be related to the presence of leukocytes in the concentrate in preparations with higher platelet concentrations. The results of clinical studies that do not control for platelet concentration need to be interpreted with caution, and future clinical studies should report the PRP volume and platelet concentration utilized.⁹⁹ Therefore, there is a need for further studies regarding the optimal concentration of PRP, whether there is a ceiling effect with different PRP concentrations, and the optimal timing of PRP application.

TABLE 6
Biologics for the Treatment of Ligament Injuries: Targeted Areas for Future Research
and Barriers to Clinical Implementation

Targeted areas

- The use of biologics to augment the healing of autografts and allografts for ligament reconstruction, specifically graft-tunnel healing and graft maturation
- Further basic science investigation of biologic augmentation of graft-tunnel healing and graft maturation to direct the development of clinical studies
- Imaging modalities to objectively evaluate graft healing in reconstruction and the effect of biologic therapies
- Feasibility of ACL repair and the optimal criteria for targeted ACL repair
- Comparative laboratory studies on scaffolds, cells, and growth factors

Barriers

- Reliance on predominantly preclinical studies to support biologic augmentation
 - Heterogeneity in characteristics of biologic therapy as well as patient population with ligament injury
-

REGULATORY ENVIRONMENT

In 1997, the FDA proposed a regulatory framework for human cellular and tissue-based products (HCT/Ps). Title 21, Part 1271 of the Code of Federal Regulations governs such HCT/Ps and their use in human recipients. These regulations have governed the expansion of scientific advancements in both the laboratory and veterinary medicine for the clinical practice of orthopaedic surgeons in the United States. Anz et al⁷ recently provided historical perspective on the regulatory process and gave examples from recent FDA rulings.

Products are regulated through the Public Health Service Act according to level of patient risk. Section 361 addresses lower-risk products and section 351 addresses higher-risk products. To qualify as low risk, products must meet all 4 of the following criteria: minimal manipulation, homologous use only, noncombination product, and nonsystemic effect. A product that fails to meet 1 or more of these criteria is subsequently classified as high risk.

Both product classes must be manufactured under good tissue practices to prevent transmission of communicable disease. Furthermore, Section 351 products must undergo a premarket approval process, in which safety and efficacy are evaluated through preclinical animal studies and human trials. Such products require a biologics license to be lawfully offered and marketed.

Minimally manipulated PRP and bone marrow aspirate are not presently regulated as HCT/Ps, although they must be appropriately registered and use good manufacturing processes. As reviewed, MSCs require culture expansion to obtain adequate numbers for implantation (this is considered more than minimal manipulation), and intra-articular injection of adipose-derived stem cells harvested via liposuction is considered nonhomologous use. These examples highlight laboratory-supported MSC treatment options that would likely be classified as high risk because they do not meet all 4 criteria for low-risk products.

These regulations are intended to ensure patient safety, but they consequently limit advancement of the field because of the complexity of the US FDA Section 351 regulatory pathway. Currently, clinical investigators have mostly pursued treatment with Section 361 products (lower risk) that comply with current regulations, as

reviewed in this article. Although legitimate cost concerns exist, in the near term, expansion of high-risk products for routine use in patient care will need to occur through the existing FDA framework.

BIOLOGIC OPTIONS TO AUGMENT HEALING IN LIGAMENT RUPTURE

The ACL has been studied extensively with respect to its anatomy, biomechanics, treatment options for rupture, and clinical outcomes and serves as a good model for the study of ligament injuries because of its high injury incidence and importance for knee biomechanics. Historically, ACL repair was initially associated with early favorable outcomes; however, midterm follow-up revealed a high incidence of recurrent symptoms and meniscal injury.³⁵ Furthermore, it has been demonstrated that a complete ACL rupture does not undergo healing (ie, restoration of functional stability) with nonsurgical treatment.¹¹

With improved understanding of anatomy as well as the development of multiple graft and fixation options, arthroscopically assisted reconstruction has become the current standard surgical treatment for active patients with ACL tears and knee instability. Aided by biologic augmentation, improved healing of reconstruction grafts and options for repair have received greater research attention in recent years with *in vitro* studies, preclinical animal models, and some early clinical studies. Strategies to improve graft healing in biologically impaired conditions may facilitate earlier and more aggressive postoperative rehabilitation programs. The following is an overview of biologic options to augment ligament healing, and Table 6 presents targeted areas for future research and barriers to clinical implementation.

ACL Reconstruction Graft Maturation

Current ACL reconstruction techniques rely on a tendon graft that undergoes a maturation process termed *ligamentization*. Arnoczky et al⁸ reported necrosis of deep-frozen allografts in a canine study, whereas necrosis was not observed in a sheep study using hamstring autografts performed by Goradia et al.³⁹ A recent systematic review

highlighted the relatively limited number of human studies on graft maturation and suggested that the process is a continuum and may take more than 2 years.²³ Slower graft maturation, such as can occur with allograft tissue, may result in ACL graft elongation or failure over time.

ACL Reconstruction Graft-Tunnel Healing

Integration of an ACL reconstruction graft within its bone tunnel is also believed to be an important aspect of ACL graft healing. Grana et al⁴¹ reported early formation of collagenous fibers that provided early fixation of a hamstring graft to bone in a rabbit model and were similar to the appearance of Sharpey fibers. The application of BMP2 to the bone-tendon interface has been reported to improve healing of the interface and improve pullout strength through improved osseous ingrowth.⁵ TGF β also enhanced bone formation within the tunnel wall at the graft-bone interface.⁹⁷ Improved means for healing of the bone-tendon interface may allow earlier rehabilitation progression and an earlier return to work and sporting activities.

Biologics and ACL Reconstruction

The use of PRP after ACL reconstruction has also been investigated. ACL reconstructions are among the most frequently performed surgeries in the United States.³⁷ Despite the reported generally good outcomes after an ACL reconstruction, patients have a 3- to 5-fold greater risk of the development of posttraumatic osteoarthritis compared with the uninjured contralateral control group.^{3,10} It has been proposed that the early administration of PRP postoperatively may accelerate or potentiate the healing cascade and lead to earlier ACL graft healing.⁸¹ In fact, the authors of a systematic review of the use of PRP postoperatively concluded that its use may have a 20% to 30% beneficial effect on earlier graft maturation.⁹⁴ In addition, the use of a PRP gel at the patellar tendon graft harvest site was found to accelerate patellar tendon donor site healing and its anti-inflammatory effects were also thought to decrease postoperative pain.²⁸

A recent study that utilized a bone-patellar tendon-bone autograft canine model reported that TGF β 1 application inhibited the natural deterioration of the ACL graft and also enhanced healing and remodeling of the tendon reconstruction graft.⁹⁸ In addition, a synergistic beneficial healing effect has been reported when TGF β 1 is used concurrently with VEGF.⁹⁵

Vascular-derived Stem Cells, Angiogenesis

Although reconstruction with a tendon graft continues to be the predominant treatment choice for ACL tears in active patients, there is laboratory evidence of an intrinsic ACL healing capability. The blood vessels in the septum between the 2 bundles of the ACL contain cells expressing CD34 and CD146 surface markers, and these cells were found to exhibit stem cell characteristics and may contribute to healing and regeneration of the injured ACL.⁶⁰ Takayama et al⁹¹ recently evaluated the effect of inhibiting

angiogenesis on ACL healing. It was found that VEGF promotes angiogenesis for ACL healing, whereas inhibiting VEGF (with soluble fms-like tyrosine kinase-1) led to reduced graft maturation and biomechanical strength.⁹¹

ACL Reconstruction Bioaugmentation With Cell Sheets

Cell sheet technology has recently been developed for stem cells for improved delivery to affected tissues. Mifune et al⁶⁴ investigated the use of a cell sheet impregnated with CD34-expressing vascular-derived stem cells obtained from the central septal region of the ACL to augment ACL reconstruction in a rat model. They reported enhancement of healing at the bone-tendon junction by the deposition of greater numbers of collagen fibers connecting the graft to the bone tunnel, quicker graft maturation, and increased ACL graft biomechanical strength compared with injection of the same cells intra-articularly. This technique has been suggested to result in improved cell incorporation into the grafted tendon compared with direct intra-articular cell injections.

ACL Repair With Biologic Augmentation: Preclinical Studies, Clinical Trial

ACL bioenhanced repair and ACL reconstruction had no biomechanical differences in a porcine study.⁹⁴ The authors of a study of 64 minipigs with 4 groups (bioenhanced ACL repair, bioenhanced ACL reconstruction, traditional ACL reconstruction, and ACL transection) reported no difference in the biomechanical properties of an ACL repair versus reconstruction.⁷⁵ Of note, there was a decreased incidence of chondral degeneration at 12 months for the bioenhanced ACL repair compared with both ACL transection and reconstruction. Supported by preclinical studies,^{75,93} Murray et al recently initiated a prospective study of bioenhanced ACL repair in a select patient group ("Bridge-Enhanced ACL Repair (BEAR) Clinical Trial"; ongoing study).

BIOLOGIC OPTIONS TO AUGMENT HEALING IN TENDINOPATHY

Tendinopathy Overview, Basic Science, and Imaging

Overview. The clinical condition of tendinopathy encompasses subjective pain and patient-reported dysfunction with objective histologically identified pathologic characteristics of tendons and has been characterized as a failed healing response with multiple suggested origins.⁵⁴ The presence of a continuum of tendon pathology has been proposed,^{24,47,62} although this concept has not been fully accepted into clinical use. Correlation of histology with the presence of pain requires further investigation, although inflammation and neurovascular ingrowth have been implicated.⁶²

Tendinopathy represents a significant proportion of overuse injuries and may lead to disability and prolonged time away from athletic training or work. Furthermore, underlying tendinopathy has been implicated in up to 97% of acute tendon ruptures.⁴⁶ An improved understanding of the basic

TABLE 7

Biologics for the Treatment of Tendinopathy: Targeted Areas for Future Research and Barriers to Clinical Implementation

Targeted areas

- Tendinopathy basic science
 - Further research to more accurately link the histological stages of tendinopathy to clinical findings so that treatment methods can be targeted to treat this pathology objectively
 - Clarification regarding the role of angiogenesis in tendon healing
- PRP in treatment of tendinopathy
 - Improved reporting of PRP contents in clinical treatment of tendinopathy
 - Further randomized controlled trials with control of PRP contents and timing of injection in the disease process
 - Effect of PRP on healing with or without leukocytes
- Stem cells in treatment of tendinopathy
 - Clarification of the mechanism of MSC therapy for tendinopathy—paracrine effect, immunomodulation, or direct engraftment
 - Clarification through laboratory and clinical methods to determine optimal timing and frequency of implantation of MSCs
 - Ability to control tenogenic differentiation of resident or implanted MSCs
 - The optimal number (and need for expansion *ex vivo*) and concentration of MSC in the treatment of tendinopathy
 - The optimal source of MSCs (specifically, bone marrow– versus adipose-derived) is unknown
 - Clinical study of adipose-derived stem cells for treatment of tendinopathy
- Diagnostic modalities
 - Continued development and standardization of objective tools for diagnosis and monitoring of treatment progress

Barriers

- Heterogeneity of clinical presentation for patients with tendinopathy
- Difficulty in developing animal model of tendinopathy
- Lack of agreement on pathophysiology of tendinopathy
- Inconclusive PRP evidence for certain pathology and anatomic sites limits incorporation into clinical practice and development of large randomized controlled trials to support use
- Regulatory environment surrounding stem cell therapy complicates development of clinical solutions

science of tendinopathy and an objective means to diagnose tendinopathy is crucial in evaluating treatment methods. The following is an overview of biologic options to augment healing in tendon disorders, and Table 7 provides a summary of targeted areas for future research and barriers to clinical implementation.

Basic Science of Tendinopathy. Tendon healing has been evaluated experimentally using transected animal tendon models and it occurs acutely in 3 overlapping phases: inflammation, proliferation, and remodeling.^{68,88} The inflammatory phase is characterized by increased vascular permeability and a local influx of inflammatory cells that release chemotactic agents to recruit blood vessels, fibroblasts, and intrinsic tenocytes. During the proliferative phase, fibroblasts produce collagen and matrix with concomitant angiogenesis. During the remodeling phase, which commences at approximately 6 weeks, total cellularity decreases and type I collagen content increases. The collagen orients more parallel to the axis of the tendon and forms cross-links with adjacent healthy matrix as the healing response matures over several months.

The healing of transected tendons has been described in a relatively clear progression of events; however, the presence of a clear progression of histological events for tendinopathy is debated. Cook and Purdam²⁴ described a continuum starting with normal tendon and progressing through reactive tendinopathy, tendon disrepair, and finally degenerative tendinopathy. The role of inflammation in early tendinopathy has been reported by Millar et al,⁶⁵ although inflammation is not believed to play a role in disease progression.⁵⁵ However,

inflammatory mediators may play a role in tendinopathy whether or not inflammatory cells are found near the lesion.⁴⁷

Reactive tendinopathy is characterized by synthesis of large proteoglycans and a subsequent increase in bound water; this results in a fusiform swelling on imaging, including ultrasonography and magnetic resonance imaging (MRI). There may be an inflammatory component with early tendinopathy.^{1,62} During the tendon disrepair stage, there is matrix disorganization and separation of collagen. Degenerative tendinopathy is characterized by areas of acellularity, apoptosis,⁵³ disorganized matrix, and areas with limited collagen.²⁴

Animal models of chronic tendinopathy have utilized induced injury from incline/decline treadmill running (“overuse” injury), partial laceration, and collagenase injection.⁹² However, models that replicate the biological processes in human chronic tendinopathy are lacking and further research is necessary.

Tendon histologic properties have been evaluated in diseased and adjacent normal tendon.⁸⁰ In tendinopathic regions, there was reported to be an increase in the ratio of collagen type III to type I fibers, buckling of the collagen fibrils in the extracellular matrix, buckling of the tenocytes and nuclei, increased lipid deposition, calcification, and decreased large-diameter fibers. No inflammatory cells were identified in the chronic tendinopathic biopsy specimens. However, a recent systematic review by Dean et al³⁰ suggested that inflammatory cells including macrophages, mast cells, and T cells were present in intact tendinopathic tissue.

The possible uses of biologic agents to enhance or restore healing in this phase will require different treatment strategies than acute or subacute tendon pathologies and thus need further investigation. In addition, improvement of animal models of tendinopathy that more closely replicate the process in humans is necessary.

Imaging Modalities for Tendinopathy. The use of noninvasive imaging modalities, including ultrasonography and MRI, allows for the potential diagnosis and monitoring of patients with specific stages of tendinopathy. Improved methods to detect pathologic changes in soft tissues are necessary to allow for the optimal timing and monitoring of treatment. Ultrasonography has been used to evaluate the patellar tendon in jumping athletes. In a prospective study of volleyball players, ultrasonography was used to correlate the onset of pain with findings of neovascularity in patellar tendinopathy.^{57,58} Studies have also focused on the use of quantitative MRI to evaluate tendon properties and specifically the effect of cyclic loading on T_2^* values.⁴⁸ The use of specific imaging protocols may ultimately allow for the assessment of tissue organization and the effects of biologics using a quantitative metric.

Use of MSCs for the Treatment of Tendinopathy

Preclinical Studies on MSCs for the Treatment of Tendinopathy. The study of the microenvironment of tendinopathy is a key factor to improve tendon healing by treatment with MSCs.¹⁷ Prolonged mechanical stimuli have been proposed as a potential mechanism of tendinopathy because it induces the production of cytokines, inflammatory prostaglandins, and MMPs as well as tendon cell apoptosis and chondroid metaplasia.^{6,88} Modification of this environment may affect the natural course of tendinopathy. Undesired proinflammatory effects may theoretically be present if the stem cells are injected too early in the injury process. However, later injection may result in a desired immunosuppressive effect and injury resolution. Further study regarding the timing of stem cell treatments for tendinopathy is required.

The equine veterinary literature serves as a good source for basic science and clinical information on the use of MSCs for treatment of tendinopathy in veterinary athletes. Often, the superficial digital flexor tendon (SDFT) in performance horses is used as a model for the human Achilles tendon. Smith et al⁸⁹ described a novel technique for treatment of SDFT pathology in a polo horse using in vitro expanded stem cells. In a more recent study, bone marrow-derived and expanded MSCs were injected into the SDFT with a randomized study in 12 racehorses with “career-ending” injuries.⁹⁰ Improved biomechanical (normalized stiffness) and histological (organization, glycosaminoglycan content) parameters were found in the MSC-treated group. Autologous MSC treatment for SDFT pathology was associated with a recurrence rate of 27% compared with a 56% recurrence rate with conventional treatment.¹⁰⁰

Clinical Studies on the Use of Stem Cells for Treatment of Tendinopathy. Stem cells have been utilized with some success in animal models for the augmentation of healing repairs of surgically created tendon defects. However, the use of stem cells for the treatment of tendinopathy in the

human clinical setting has been slow in development. A search of ClinicalTrials.gov (August 1, 2015) revealed only 1 study (although not yet recruiting) investigating the use of stem cells for treatment of tendinopathy.

Evidence for the use of adipose-derived stem cells for tendinopathy is limited, and a recent search of PubMed (August 1, 2015) using the search term “adipose stem cells tendinopathy” returned only 5 relevant articles; 3 were on the subject of equine tendinopathy, 1 focused on “lateral epicondylitis,” and 1 was a systematic review from 2010. Further research is required to more accurately link the histologic stages of tendinopathy to clinical findings so that treatment methods can be targeted to treat this pathology objectively.

Tendon-Derived Cells. Expansion of harvested tenocytes with subsequent reimplantation has been described, specifically with the patellar or palmaris longus tendons as the source.¹⁰⁰ Autologous tenocyte implantation has been evaluated in a clinical trial in the Netherlands for Achilles tendinopathy (see ClinicalTrials.gov), although results have not yet been published in the peer-reviewed literature.

Use of PRP for the Treatment of Tendinopathy

Preclinical Studies on PRP for Modulation of Inflammatory Processes. PRP has also been evaluated for its role in the reduction of inflammatory mediators and its ability to improve healing in tendinopathy. An in vitro experiment on rabbit tendon cells and an in vivo experiment on a mouse Achilles tendon injury model were performed to investigate the effect of PRP containing hepatocyte growth factor (HGF).¹⁰¹ Investigators reported that PRP with HGF resulted in the suppression of cyclooxygenase-2 (COX-2) expression and reduced prostaglandin E2 (PGE2) production, both known inflammatory cytokines, supporting its role as an anti-inflammatory mediator. PRP is also reported to have a positive effect on healing in an in vitro study using rabbit patellar tendon stem cells.¹⁰² In this study, an anabolic effect was found with PRP, resulting in increased collagen production and number of activated tenocytes. HGF, along with PRP, was also reported to suppress tendon inflammation and to decrease PGE2 production. Studies have also reported that LR PRP lead to an increased acute inflammatory response, whereas LP PRP resulted in less inflammation.^{16,32} In concert with the in vitro PRP anti-inflammatory effects reported to date, further investigation is needed to ascertain whether these effects can decrease pain and improve function clinically in patients with tendon pathology. In addition, the use of a PRP gel at the patellar tendon graft harvest site was found to accelerate patellar tendon donor site healing and its anti-inflammatory effects were also thought to decrease postoperative pain.²⁸

Clinical Use of PRP for Tendinopathy. Anatomic sites of tendon overuse injuries have been described, including most commonly the Achilles tendon, patellar tendon, and wrist extensors,⁷⁶ although the posterior tibial tendon, iliotibial tract, hamstring tendons, and rotator cuff tendons may also be involved.⁵⁶ The use of PRP to treat tendinopathy has been reported in prospective clinical studies,

including those on lateral epicondylar tendinopathy,⁶⁷ Achilles tendinopathy,²⁹ and patellar tendinopathy.^{34,79}

The clinical condition of lateral epicondylar tendinopathy has been histologically described as angiofibroblastic dysplasia.⁴⁹ Mishra et al⁶⁷ performed a double-blind randomized controlled trial of needling with or without leukocyte-enriched PRP for chronic lateral epicondylar tendinopathy. At 24 weeks, the investigators found significant improvement for the PRP-treated cohort compared with the control group for both lateral elbow tenderness and overall treatment success.

Midportion Achilles tendinopathy can affect both running athletes and the sedentary population. The use of PRP to augment the treatment of chronic Achilles tendinopathy was evaluated by de Vos et al.²⁹ All study participants underwent an eccentric exercise program and investigators randomized 27 patients to the PRP group and 27 to the placebo (saline) group. There was no reported difference in pain or activity scores between the 2 groups.

Dragoo et al³⁴ studied the role of PRP in the treatment of patellar tendinopathy in a recent double-blind randomized control trial. Standardized eccentric exercises were performed by both groups; one group received dry needling alone and the other received dry needling along with LR PRP. The PRP group had greater early clinical improvement at 12 weeks.

A likely cause for the reported inconsistent effect of PRP on healing tissues is that studies have varied in the concentration of platelets, leukocytes, and other factors. Future studies need to better define the type of PRP and the leukocyte concentration to best determine its ability to augment tissue healing.⁶⁶

PRP likely has a role in the treatment of chronic tendon pathology, although the indications are still evolving. PRP may also have potential to enhance the healing of acute soft tissue injuries, although rigorous scientific evidence is limited.⁶³ Its use early after injury may be beneficial because of the presence of growth factors, such as platelet-derived growth factor (PDGF) for angiogenesis and TGF β for collagen synthesis, although the optimal timing is not yet known. The optimization of the use of PRP in vivo and the use of adjuvant growth factors requires further advanced and collaborative scientific investigation among research centers. Clarifying the role of PRP in accelerating ligament and tendon healing is important and an objective measure of its ability to improve tissue structure and overall joint function is essential. Customization of PRP for specific pathology and specific patient populations warrants further study, and it is likely that this will allow realization of more clearly defined indications for use.

CONCLUSION

The continued laboratory and preclinical investigation of biologic treatments for sports injuries has led to increased clinical use of biologics by orthopaedic surgeons. However, significant knowledge gaps still exist and must be addressed before expanded clinical use. Expanded use of MSCs in the clinical setting will depend on a continued dialogue between

clinicians, scientists, and regulators. To support clinical use of PRP in the treatment of acute and chronic soft tissue injuries, further clarification and standardization of its contents and the optimal use for various clinical conditions will be essential. Although some biologic treatments have shown great promise for benefiting tissue healing, many areas remain unstudied and the true efficacy of specific treatments must now be clarified and clinical indications defined. Further rigorous and objective studies are necessary before widespread clinical use.

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