Bioaugmentation in the surgical treatment of anterior cruciate ligament injuries: A review of current concepts and emerging techniques

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Abstract

Injuries involving the anterior cruciate ligament are among the most common athletic injuries, and are the most common involving the knee. The anterior cruciate ligament is a key translational and rotational stabilizer of the knee joint during pivoting and cutting activities. Traditionally, surgical intervention in the form of anterior cruciate ligament reconstruction has been recommended for those who sustain an anterior cruciate ligament rupture and wish to remain active and return to sport. The intra-articular environment of the anterior cruciate ligament makes achieving successful healing following repair challenging. Historically, results following repair were poor, and anterior cruciate ligament reconstruction emerged as the gold-standard for treatment. While earlier literature reported high rates of return to play, the results of more recent studies with longer follow-up have suggested that anterior cruciate ligament reconstruction may not be as successful as once thought: fewer athletes are able to return to sport at their preinjury level, and many still go on to develop osteoarthritis of the knee at a relatively younger age. The four principles of tissue engineering (cells, growth factors, scaffolds, and mechanical stimuli) combined in various methods of bioaugmentation have been increasingly explored in an effort to improve outcomes following surgical treatment of anterior cruciate ligament injuries. Newer technologies have also led to the re-emergence of anterior cruciate ligament repair as an option for select patients. The different biological challenges associated with anterior cruciate ligament repair and reconstruction each present unique opportunities for targeted bioaugmentation strategies that may eventually lead to better outcomes with better return-to-play rates and fewer revisions.

Keywords

ACL reconstruction, ACL repair, ACL surgery, anterior cruciate ligament, biological augmentation, knee ligament reconstruction, orthopedics/rehabilitation/occupational therapy

Introduction

Despite many technological advancements in the dynamic field of orthopedic sports medicine, emergent data suggest that the long-term outcomes following the surgical treatment of anterior cruciate ligament (ACL) injuries may not be as optimistic as previously thought. There are an estimated 200,000 ACL injuries annually, of which up to 150,000 are treated surgically.[1–3] ACL reconstruction (ACLR) has traditionally been recommended as protective against subsequent meniscal injury and cartilage damage, and ultimately osteoarthritis.[2,4–6] In contrast with a greater than 90% success rate[7] and 67% good or excellent outcomes,[8] more recent publications have found a higher rate of revision following ACLR, ranging from 10% to 15%,[9] and similar rates of radiographic osteoarthritis as with nonoperative management at long-term follow-up.[10] This has inspired many to search for opportunities for improvement in the surgical management of these common athletic injuries.

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Historically considered as an unreliable treatment option associated with high failure rates and complications related to intra-articular immunogenic reactions,[11] some authors have again begun to explore ACL repair for certain patients.[12–14] Still others have sought ways to enhance the gold-standard of reconstruction.[15–18] Both procedures present unique challenges for which an increasing number of new biologic augmentation and tissue engineering products and techniques have been developed. However, while the number of products available in this space continues to grow at an exponential rate, there is little guidance available regarding optimal indications, and often insufficient evidence to support their use.

This article reviews the principles of tissue engineering as applied to orthopedic sports medicine, including the biological, biomechanical, and materials science factors involved in various bioaugmentation strategies, with a focus on improving outcomes following the surgical treatment of ACL injuries with repair or reconstruction. Two of the authors (J.D.L. and A.R.H.) searched PubMed/MEDLINE with the terms "anterior cruciate ligament," "surgery," "repair," "reconstruction," "biologic," and "augmentation," combined with the Boolean operators "AND" and "OR." A final search was performed on 1 October 2019.

Principles of tissue engineering in surgical treatment of ACL tears

Cellular elements

The optimal cellular response following surgical treatment varies depending on the procedure performed. Successful healing following ligament repair requires the presence of cells at the repair site that have the ability to proliferate in sufficient numbers and elaborate the extracellular matrix (ECM) that gives the ligament its biomechanical properties. Healing after ACLR is dependent upon both graft remodeling and soft-tissue grafts, for integration of the grafted tendon–bone interface.[19]

Following ACLR, the grafted tendon continues to mature and integrate via progressive cellular phases—acute inflammatory, revascularization, recellularization, and tissue remodeling phases, respectively.[20,21] Through the process of "ligamentization," the graft remodels and matures, eventually taking on physical and mechanical characteristics that resemble the native ligament more than the original grafted tendon.[22–25] This begins with neovascularization, followed by fibroblast repopulation.[24,25] Early fibroblasts are randomly arranged and disorganized, and display cellular characteristics that typify high levels of metabolic activity; however, with remodeling, these become longitudinally aligned.[26] Disorganized collagen fibrils predominate earlier in the process, but these too become longitudinally organized with maturation.[22,26] When newly formed connective tissue predominates but has yet to undergo longitudinal reorganization, the graft is mechanically weak and prone to failure.[27,28]

In addition, the tendon–bone interface remains relatively unstable during the healing process. In the native ACL, a fibrocartilaginous tissue exists at the bone–ligament interface.[29,30] Following ACLR with soft-tissue grafts, the bone–tendon junction matures through many of the same stages as the graft (i.e. inflammation, proliferation, and matrix remodeling), but heals through formation of fibrous scar-like tissue that does not undergo substantial remodeling.[19,31,32] This creates a relative weak point that can contribute to rerupture.[19,29,32,33]

The most widely explored cellular elements in the treatment of ACL ruptures include stem cells and platelet therapies. A population of perivascular tissue-specific stem cells resides in the septum between the two bundles of the ACL with fibroblastic potential, which may indicate an innate healing capacity.[34] Nevertheless, the limited bioavailability of these cells combined with the fact that under current Food and Drug Administration (FDA) regulations ex vivo expansion is not permitted has limited their overall use thus far.[35] As an alternative, mesenchymal stem/stromal cells (MSCs) have been widely explored in musculoskeletal medicine. Compared with ACL-derived stem cells, MSCs have shown a relative ease of isolation, multipotency, and relatively high proliferative capacity.[36,37] These multipotent tissue-adherent cells have the ability to differentiate into osteogenic, adipogenic, and chondrogenic lineages.[38] MSCs have also been shown to have fibroblastic capacity, and may therefore also have a role in tendon and ligament healing.[39–41] Yet, the FDA restrictions on ex vivo expansion also apply to MSCs.[35] Therefore, most of the clinical work involving MSCs is limited to the use of bone marrow aspirates and similar products that meet the standards of minimal manipulation, but which provide a highly variable and unreliable source of stem cells.[27,42–45]

In addition to MSC bioaugmentation in ACLR, evidence has been presented in the orthopedic sports medicine literature regarding the utility of platelet-rich plasma (PRP) in soft-tissue healing. PRP is an autologous blood product which has long been implemented in the treatment of degenerative cartilage as well as tendon lesions due to its multiple growth factors and bioactive molecules allowing for tissue healing and vasculogenesis.[46–48] Due to its availability and ease of harvesting, PRP is a versatile healing agent that can be utilized through intra-articular injections or through scaffolding aimed at increasing graft healing. PRP has many potential benefits in ACL surgery, including anti-inflammatory properties, growth factors, and bioactive substances.

Andriolo et al.[49] conducted a systematic review to examine the utility of PRP in ACL graft ligamentization and inflammatory modulation, and identified limited evidence from multiple studies to support a positive impact on accelerating the graft maturation process and incorporation, but significant variability regarding dose and concentration. This was investigated in a preclinical study by Fleming et al.,[50] which sought to answer whether an increasing platelet concentration in an ECM scaffold would improve graft biomechanical properties and/or

Table 1. Growth factors with functional relevance to the bioaugmentation of anterior cruciate ligament repair and reconstruction.

Growth factor	Functional roles				
	Cell proliferation	Collagen synthesis	ECM production	Neovascularization	Cell migration
EGF	+	+	–	–	–
FGF	+	+	+	+	–
IGF1	+	+	–	–	+
GDF	+	–	–	–	+
PDGF	+	+	+	+	+
VEGF	–	–	–	+	+
TGFβ	+	+	–	–	–
BMP2	+	+	–	–	–

ECM: extracellular matrix; EGF: endothelial growth factor; FGF: fibroblast growth factor; GDF: growth differentiation factor; IGF: insulin-like growth factor; PDGF: platelet-derived growth factor; VEGF: vascular endothelial growth factor; TGF: transforming growth factor; BMP: bone morphogenetic protein.

decrease cartilage damage after ACLR. The study consisted of 55 minipigs randomized into five treatment groups: untreated ACL transection, conventional ACLR, and reconstructions with physiologic (1×) and supraphysiologic (3× or 5×) concentrations of PRP. Biomechanical properties, anteroposterior knee laxity, graft histology, and cartilage integrity were measured at 15 weeks after surgery. Grafts treated with physiologic concentration (1×) of platelets resulted in an increased stiffness over control ($p = .03$), yet there was no significant increase in graft linear stiffness at 3× or 5× ECM-platelet composite groups. Mean macroscopic cartilage grades were determined using bundle orientation and crimp appearance. According to cartilage grading, there was significantly improved cartilage appearance in the bio-enhanced ACL when compared to control, but there was no difference among the 1×, 3×, or 5× groups.

Growth factors

Growth factors have been shown to both play an important role in differentiation of tendons and ligaments during development and the healing process following injury by increasing cellularity and volume of tissue at repair sites. Broadly, a growth factor can be defined as a protein that affects cell migration, proliferation, and differentiation.[51] Growth factors have short half-lives and diffuse slowly through the ECM to act locally.[52] Cell proliferation, ECM synthesis, vascularization, as well as mechanical properties can be dramatically influenced by the presence of growth factors.[53,54]

While the exact signaling mechanisms involved in ligament development and repair have yet to be completely characterized, many growth factors known to have mitogenic effects on musculoskeletal tissues have been extensively investigated. In studies involving the roles of growth factors in ACL tissue engineering, epidermal growth factor (EGF), fibroblast growth factor (FGF), growth and differentiation factor (GDF), insulin-like growth factor (IGF), platelet-derived growth factor (PDGF), and transforming growth factor-beta (TGFβ) have all been shown to increase cell proliferation, fibroblastic differentiation, and/or matrix production Table 1.[55–57] In particular, TGFβ may help prevent graft deterioration and enhance osseous ingrowth at the tunnel wall.[58–60]

Angiogenesis and osteogenesis are integral to tendon–bone healing following ACLR. If perfusion is delayed following a reconstruction, the grafted tendon may degenerate.[19] If tendon–bone healing is suboptimal, biomechanical strength of the grafted tendon may be sacrificed.[27] Vascular endothelial growth factor (VEGF) and bone morphogenetic protein 2 (BMP2) have both been studied in ACLR. VEGF has been shown to stimulate angiogenesis as well as act as a chemotactic agent for macrophages and granulocytes.[61] In animal studies, VEGF was shown to promote angiogenesis in the grafted tendon following ACLR.[62] VEGF has been shown to exhibit a synergistic effect on tendon healing in concert with TGFβ.[63] In a study assessing ACL healing, VEGF was found to promote angiogenesis that aided in the healing process.[64]

BMP2 has been shown to induce MSC proliferation, osteogenic differentiation, chondrogenic differentiation, as well as collagen production.[65–68] BMP2 has demonstrated beneficial effects in fracture healing in multiple studies.[69,70] An important aspect in the healing process of ACLR with soft-tissue grafts is the integration of the grafted tendon within its bone. BMP2 has improved healing of the tendon–bone interface through improved osseous ingrowth.[71] Despite the enthusiasm surrounding these findings, these discoveries have proven difficult to implement in a clinically meaningful way.[72]

A challenge arises in that there are generally few cells at the repair sites with tendons and ligaments that preclude the growth factor ability to sufficiently improve strength or stiffness. With the recent advances in biomaterials and molecular biology, more investigators are incorporating growth factors into biomaterials for controlled release or using gene therapy techniques to upregulate cellular production of growth factors. The combined delivery of growth factors with stem cells at the time of surgery and maintenance at the repair/

Table 2. Scaffolds used in the bioaugmentation of anterior cruciate ligament repair and reconstruction.

Material	Product	Manufacturer	Structural	Bioinductive	Results
Biologically derived					
Human dermis ECM	GraftJacket	Wright Medical	++	+	No ACL-specific results or outcomes Increased load-to-failure force in biomechanical cadaveric Achilles tendon (Barber et al.)[74] Positive effect on graft incorporation on postoperative MRI following massive RCR (Bond et al.)[75]
	Allopatch HD	MTF Biologics	+	+	Limited relevant clinical or preclinical data available
	Dermaspan	Biomet	++	+	Limited relevant clinical or preclinical data available
Collagen	Integra	LifeSciences	+	+	Limited relevant clinical or preclinical data available
	TissueMend	Stryker	++	+	No ACL-specific results or outcomes Superior stiffness in biomechanical testing compared with GraftJacket (Song et al.)[76]
	Zimmer Patch	Zimmer	+	+	No ACL-specific results or outcomes Durable graft in RCR repair augmentation (Badhe et al.)[77]
	Regeneten	Smith & Nephew	–	++	No ACL-specific results or outcomes Rapid recovery and significant ASES pain score improvement for partial-thickness RCR (Schlegel et al.)[78]
Silk	SeriACL	Serica Technologies	++	+	Silk scaffold supported collagen growth and maintained stability without generating immune response (Altman et al.)[79] Higher tensile strength than collagen; promotes adult stem cell growth (Altman et al.)[80]
Synthetically derived					
Polyethylene terephthalate (PET)	Leeds-Keio	Xiros	++	–	No ACL-specific results or outcomes Superior clinical results following augmented subscapularis transposition (Tanaka et al.)[81]
	Poly-Tape	Yufu Itonaga	++	–	Limited relevant clinical or preclinical data available
Poly-L-lactic acid	X-repair	Medtronic	+	–	No ACL-specific results or outcomes 25% increase in RCR repair strength over control in animal model (Koh et al.)[82]
Polyurethane urea	Artelon	Artimplant	+	–	No ACL-specific results or outcomes Superior healing and higher patellar tendon repair strength in animal model (Gersoff et al.)[83] Some concern about adverse intra-articular reactions in hand surgery in human subjects (Robinson and Muir)[84]
	SportMesh		+	–	No ACL-specific results or outcomes Significant clinical improvement in augmented degenerative subscapularis repairs (Petriccioli et al.)[85]

ACL: anterior cruciate ligament; ASES: American Shoulder and Elbow Surgeons; ECM: extracellular matrix; RCR: rotator cuff repair; MRI: magnetic resonance imaging; PET: positron emission tomography.

reconstruction is likely to be a key element in the next generation of targeted bioaugmentation techniques.[19,27]

Scaffolds

A scaffold is an artificial structure capable of supporting three-dimensional tissue formation that allows cell attachment and migration, delivery of biochemical factors, and diffusion of vital cell nutrients and expressed products.[51] An ideal scaffold possesses the following characteristics:[73]

1. Three-dimensionality and high porosity with an interconnected pore network for cell growth and flow transport of nutrients and metabolic waste;

2. Biocompatibility and bioresorbability with a controllable degradation and resorption rate to match cell/tissue growth in vitro and/or in vivo;
3. Suitable surface chemistry for cell attachment, proliferation, and differentiation;
4. Mechanical properties to match those of the tissues at the site of implantation.

A variety of biologically and synthetically derived materials have been explored as scaffold materials, with variable bioinductive and mechanical properties.[40] As summarized in Table 2, some popular scaffolds have been developed primarily to contribute mechanical stability to the repair or reconstruction construct,[86] though it is important to note that

longer-term evaluations of clinical outcomes with this technique remain limited. Several examples include GraftJacket (collagen; Wright Medical, Arling, TN, USA), Integra (collagen; LifeSciences Corporation, Plainsboro, NJ, USA), TissueMend (collagen; Stryker Orthopedics, NJ, USA), and Zimmer Patch (collagen; Tissue Science Laboratories; Covington, GA, USA). Others were designed to optimize and enhance the healing process:[86,87] Regeneten (collagen; Smith & Nephew, Andover, MA, USA), X-repair (poly-L-lactic acid; Synthasome, CA, USA), and Artelon (polyurethane urea; Artimplant, AB, Sweden).

The functional role of tendons and ligaments is supported by a highly organized structure of type I collagen. The collagen that develops in the repair and remodeling stages of tendon and ligament healing is less organized than that in the uninjured tissue, resulting in inferior mechanical properties and an increased risk for reinjury.[88,89] Accordingly, multiple collagen-based products have been examined as scaffolds to enhance mechanical stability.[90–92] While collagen has the advantage of acting as a biocompatible scaffold, several studies have demonstrated a lack of mechanical strength beyond 6 weeks.[92,93] Similar to collagen, silk has the advantage of being biocompatible and demonstrates adequate tensile strength. Silk is also a biodegradable material that undergoes proteolytic degradation within 2 years. The major drawbacks associated with silk include limited cell adhesion and immunogenic responses to its sericin coating.[40] In contrast, hyaluronic acid lacks the mechanical properties of collagen- and silk-based products, but is a biocompatible component of the ECM.[37] Similarly, chitosan, a biocompatible polysaccharide that can come in sponge or hydrogel form, is chemically modifiable and has antimicrobial properties. Chitosan too, lacks mechanical strength and experiences limited cell adhesion.[94] Alginate is another biocompatible polysaccharide that has the ability of encapsulating cells. It, too, lacks mechanical strength.[95] Poly-L-lactic acid is a biocompatible, biodegradable material that has been used in dissolvable stitches and other implants. It achieves better cell adhesion than other material and has a slow degradation rate. Its drawbacks include that it is biologically inert and creates an acidic degradation byproduct.[96]

More recently, strategies have been implemented to mitigate the inherent weaknesses of these scaffolds and maximize their strengths. The use of ultraviolet (UV) light and chemical reagents to create a cross-linked design has been shown to improve the mechanical properties of collagen scaffolds.[97] Unfortunately, the mechanical strength achieved with these techniques remain less than ideal.[90,98] A collagen–silk composite was shown to enhance the mechanical strength of the material to near-native ligament levels, but has yet to be examined in clinical trials.[99]

Mechanical stimuli

It is well documented that movement and dynamic loading are integral to maintaining the necessary mechanical properties of ligaments and tendons. Mechanical stimuli generate a host of changes in cellular functionality, tissue properties, and regenerative reactions, resulting in changes in cell differentiation and ECM production.[100] Even in the absence of growth factors, MSCs have been shown to differentiate into fibroblast-like cells in response to mechanical stimuli. Increases in cell density as well as type I and type III collagen were demonstrated in MSC-loaded collagen constructs exposed to mechanical stimuli.[101] The exact timing, strength, and direction of mechanical stimuli required for optimal cellular response is the subject of ongoing research.[43] A particularly interesting study found that mechanical stimuli initiated immediately after MSC-seeding impaired generation of type I collagen and fibronectin, while stimuli in the form of 45° rotations or static tension applied following growth factor-induced peak stem cell proliferation led to increased generation.[102] Studies have shown that cells respond to mechanical stimuli by initiating integrin-mediated focal adhesions and cytoskeleton deformation.[103,104]

Mechanical factors that encompass stiffness of the substrate, surface topography, and extracellular forces can all have significant effects on cellular function and activation of specific pathways.[40] In order to determine the optimal mechanical stimulation regimen for a specific tissue, research must be directed toward understanding the mechanical pathways involved in the development and maintenance of that native tissue. Further investigation is required to determine what, if any, mechanical stimulation is required prior to implantation of bioengineered tissue replacements in vivo, where they will be subjected to physiological mechanical forces.

Surgical augmentation strategies

The decision to incorporate bioaugmentation into surgical treatment should be targeted at overcoming specific biological or biomechanical obstacles. The indiscriminate use of bioaugmentation is not likely to contribute to a successful intervention and will be costly.[27,42] The surgical treatment of ACL ruptures provides a useful example of this principle, as repair and reconstruction each present unique biological and biomechanical challenges. In the setting of repair, the surgical construct is weakest at the time of surgery. Thus, one goal of augmentation might be to provide an appropriate mechanical environment for the early healing of the ligament during its weak stage. In addition, the harsh intra-articular environment of the knee in which repair site is bathed in synovium with poor access to vascularly delivered cells and growth factors must be overcome. By contrast, the goals of bioaugmentation in the setting of reconstruction may be directed toward achieving better tunnel healing, graft incorporation, and neovascularization, as well as possibly enhancing stability during the weakest phase of remodeling and ligamentization.

Augmentation of ACL repair

In order to overcome the harsh intra-articular environment of the knee in which the ACL repair site is bathed in synovial

fluid with poor access to the cells and growth factors required for healing, some authors have examined ways in which these elements can be incorporated at the repair site at the time of surgery and maintained long enough to contribute to healing. Some of the earliest approaches utilized hyaluronic acid carriers[105] and collagen-based matrices[14,106–109] Interestingly, the effectiveness of collagen scaffolds appears to be enhanced by the presence of platelets.[107–110] The addition of PRP to collagen-based scaffolds in platelet concentrations similar to whole blood may also deliver and maintain beneficial growth factors like PDGF, TGFβ, and VEGF, that are beneficial for ligament healing.[50,111] In animal studies, ACL repair augmentation with collagen scaffolds seeded with MSCs has shown a superior regenerative capacity over isolated repair and repair with the collagen patch alone.[112]

Human amniotic membrane tissue has been studied in its use to improve wound healing, burns, and reduce scarring and inflammation associated with ocular repair and periodontal surgery.[113,114] More recently, the use of amniotic membrane tissue for ligament and tendon repair has been explored. The basement membrane of the amnion is the thickest in the human body, resulting in high mechanical strength, while its ECM acts as a scaffold that facilitates stem cell adhesion, proliferation, and differentiation.[9] In addition, the amnion secretes a variety of growth factors that aid in the healing process, including PDGF, IGF, TGFβ, EGF, FGF, and also provides a reservoir of pluripotent stem cells.[9,115] Numerous animal studies have reported success with the use of amniotic tissue in tendon repair.[116,117] Several clinical studies have reported preliminary results for extra-articular applications, including tendon repair in foot and ankle procedures.[113,118,119] However, at this time descriptions of this technology in the setting of ACL surgery are limited to the setting of reconstruction, which is addressed in more detail in the following section.

The Bridge-Enhanced ACL Repair (BEAR) procedure developed by Murray et al.[12] combines suture repair of the ligament with implantation of a bioinductive scaffold between the two torn ends of the ligament. The BEAR scaffold is made of ECM proteins, including collagen. The scaffold is also unlinked and has a relatively low DNA content, which may lead to a decreased immunogenic response to its implantation. Autologous blood is added to the scaffold and is intra-articularly held in place within the knee where the blood cells stimulate the healing process of the ligament.

In the first in-human study, the BEAR technique was compared with ACLR with hamstring autograft ACLR in pediatric patients. All 10 of the patients in the BEAR group showed a continuous ACL or intact graft on magnetic resonance imaging (MRI) at 3- and 6-month follow-up in addition to increased hamstring strength at 3 months (mean ± SD: 77.9% ± 14.6% vs 55.9% ± 7.8% of the contralateral side; $p < .001$). The authors concluded that the use of the BEAR technique was associated with an adverse event rate low enough to warrant a high-volume study.[12] At 2 years, there

were no graft or repair failures. The International Knee Documentation Committee (IKDC) subjective scores in both groups improved significantly from baseline but were similar in the BEAR and ACLR groups at 1 and 2 years. An IKDC objective score of A (normal) was found in 44% of the patients in the BEAR group and 29% of the patients in the ACLR group at 2 years. KT-1000 testing demonstrated a side-to-side difference that was similar in the two groups at 2 years. Functional hop testing results were similar in the two groups at 1 and 2 years after surgery. Hamstring strength indices measured by dynamometer were significantly higher at all time points in the BEAR group than in the hamstring autograft group with 98.6% versus 56.3% ($p < .001$).[120]

The dynamic intraligamentary stabilization (DIS) technique for ACL repair was developed to provide a mechanical environment that protects the early repair while providing mechanical stimuli to promote healing.[121,122] However, relatively high rates of complications have been reported with DIS alone.[14,123,124] Evangelopoulos et al.[14] compared the results of DIS ACL repair with and without a protective bilayer collagen I/III membrane isolating the repair site from the synovial environment, thus combining mechanical stimulus and scaffolding elements of tissue engineering. They observed a significantly higher rate of complications in the collagen-free repair-only group (78.8%) compared with the membrane group (8.7%) ($p = .002$), and noted that the addition of the collagen membrane was the only independent prognostic factor associated with fewer complications (OR 8.0; 95% CI, 2.02–32.2; $p = .003$).[14] In a preclinical laboratory study, Gantenbein et al.[41] reported successful adherence and proliferation of ACL-derived tenocytes and MSCs on porcine collagen bilayer matrix (Chondro-Glide, Geistlich Pharma, Wolhusen, Switzerland) and bovine biphasic collagen-chondroitin sulfate matrix (Novacart, Tetec, Reutlingen, Germany). Future work will likely explore ways in which the addition of cellular elements and growth factors may be incorporated with scaffolds and mechanical stimuli in the next generation of augmentation strategies, and which patients are likely to benefit most from these techniques.

Augmentation of ACLR

Targets for augmentation of ACLR include facilitating graft-to-bone healing, optimizing the ligamentization process, and providing additional stability while the graft is transiently weak during remodeling. A number of growth factors and bioactive molecules are found in several platelet preparations such as PRP, fibrin clot, and autologous conditioned serum.[28] Several of these, including PDGF, VEGF, and TGFβ, have been implicated in both graft-to-bone healing and graft maturation and remodeling. Platelet preparations have been the subject of multiple clinical studies attempting to augment these processes, but the results remain inconsistent and inconclusive.

In small prospective randomized controlled studies, local administration of PRP gel to the graft and tunnels intraoperatively has been associated with superior healing characteristics on postoperative MRIs when compared with controls.[125–127] Radice et al.[125] reported that reconstructions augmented with PRP achieved intra-articular segment signal homogeneity on T1- and T2-weighted MRI sequences in 48% of the time required by the control group ($p < .001$), suggesting that PRP may have accelerated the graft maturation process. Vogrin et al.[127] found a significantly higher level of vascularization on contrast-enhanced MRI in the osteoligamentous interface of the PRP group (0.33 ± 0.09) when compared with the control group (0.16 ± 0.09) ($p < .001$) at 4–6 weeks. Likewise, Rupreht et al.[126] observed findings consistent with increased vascular density and microvessel permeability in the proximal tibial tunnel at 1 ($p = .019$) and 2.5 months ($p = .008$) postoperatively, suggesting a positive impact on graft-to-bone healing and incorporation. Seijas et al.[128] obtained similar results in a randomized trial with nonselective intra-articular administration of PRP injected percutaneously into the suprapatellar space following portal closure, with significantly higher stages of remodeling seen on postoperative MRIs at 4 ($p = .003$), 6 ($p < .001$), and 12 months ($p = .354$). By contrast, Orrego et al.[129] observed an isolated enhancing effect on the graft maturation process without a difference at the graft–bone interface with application of a platelet concentrate intraoperatively. Vadalà et al.[16] found that direct administration of PRP into both femoral and tibial tunnels was not effective in accelerating graft to bone integration or preventing tunnel enlargement. Mirzatolooei et al.[130] reported no significant difference in tunnel widening between PRP injection groups and controls on postoperative advanced imaging or any significant difference in laxity on clinical examination at 3 months. In a randomized controlled trial with 150 patients, PRP administration was associated with a reduction in swelling 24 h after surgery, but otherwise no difference in the IKDC scores or radiologic graft healing between PRP and control groups 1 year after surgery.[131] Komzák et al.[132] found no difference in the functional scores between test subjects and controls in a 40-patient prospective study assessing the effect of PRP on graft healing.

Given the relative non-specificity and mixed clinical results of platelet-based therapies, several authors have considered alternative more targeted techniques. Iorio et al.[133] conducted a randomized controlled trial with 40 patients examining the clinical and radiographic effects of hamstring autograft augmentation with nanohydroxyapatite to facilitate graft-to-bone healing. Lysholm, Tegner, and IKDC scores, as well as KT-1000 arthrometer readings, did not differ significantly between the experimental and control groups, though radiographic parameters associated with graft strength, interface incorporation, and bony remodeling did display a tendency toward better results with nanohydroxyapatite augmentation. In two separate randomized controlled trials with

minimum 2-year follow-up, Mutsuzaki et al.[134,135] reported superior results following ACLR with calcium phosphate-hybridized hamstring autograft. Significantly better Lysholm scores at 2-year follow-up were seen with calcium phosphate-hybridized hamstring autograft (96.9 ± 4.3) compared with controls (91.7 ± 13.3), ($p = .021$), as well as significantly less laxity on KT-1000 arthrometer testing at 1 and 2 years postoperatively (1.0 ± 2.0 mm vs 1.9 ± 1.6 mm ($p = .023$) and 1.6 ± 2.1 mm vs 2.6 ± 2.4 mm ($p = .034$), respectively), and significantly less bone tunnel enlargement in both the femur ($p = .043$) and tibia ($p = .042$).[134] Subsequently, calcium phosphate hybridization was shown to prevent bone tunnel enlargement in anatomic hamstring autograft ACLR,[135] though the clinical ramifications of this finding remain uncertain.

As with repair, the exposed nature of the intra-articular portion of the ACLR graft has led some authors to speculate that the addition of a scaffold may improve the efficacy of bioaugmentation with growth factors and platelet preparations. For instance, porous collagen scaffold carriers may reduce plasmin-mediated degradation of fibrin in PRP.[136] Berdis et al.[137] recently reported results for 109 knees in 101 adolescent patients in whom hamstring ACLR was performed with bioaugmentation with PRP contained in a porous bovine collagen matrix carrier (TenoMend; Exactech, Ramsey, NJ, USA). A total of 132 patients (92%) returned to their preinjury level of competition, while 7 patients sustained a reinjury necessitating revision surgery (5%). They felt that these results compared favorably with the 25% rate of reinjury and revision among pediatric and adolescent athletes reported elsewhere in the literature.[137,138] One patient evaluated with second-look arthroscopy for a new injury at 7 months after the initial reconstruction demonstrated complete ligamentization and neovascularization of the graft (Figure 1). As noted above, augmentation with amnion-based matrices may provide an alternative to collagen scaffolds that already contain beneficial growth factors and bioactive substances.[116] Woodall et al.[139] recently described a technique for augmentation of soft-tissue ACLR using Amnion Matrix Thick graft (Arthrex, Naples, FL, USA). Lavender and Bishop[140] have taken this a step further, adding a bone marrow composite graft to the tunnels and injecting the amnion-wrapped graft with bone marrow concentrate, and finally augmenting the construct with a suture tape brace. A small clinical trial to assess ACLR augmented with an amnion wrap and bone marrow aspirate was registered in September 2017,[141] but otherwise no outcomes have been reported for these reconstruction bioaugmentation techniques.

Internal brace augmentation

Although knee bracing postoperatively has been used in an effort to provide appropriate stability and prevent reinjury after the surgical treatment of ACL injuries,[142,143] there has
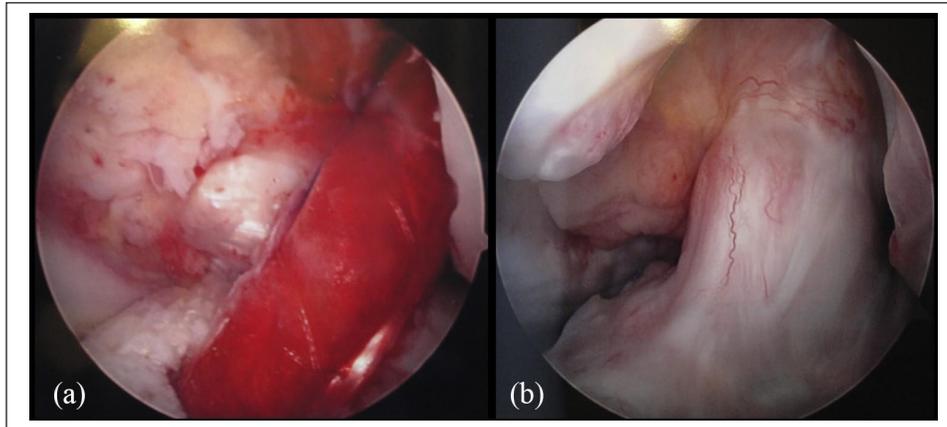
Figure 1. (a) Hamstring autograft anterior cruciate ligament reconstruction augmented with porous bovine collagen matrix carrier and platelet-rich plasma, according to the technique described by Berdis et al.[137] (b) The same patient underwent repeat arthroscopy at 7 months after the reconstruction due to another injury. The graft was found to have neovascularized and completely remodeled. Adapted with permission from Berdis et al.[137]

recently been increased attention on suture augmentation or internal bracing in the repair and reconstruction of many ligaments.[144–146] Suture tape augmentation has also been used in the setting of ACL repair[147,148], reconstruction[149] and even as a method of revising reconstructions.[150] Such constructs have been proposed to confer additional stability during healing (in the case of repair) and while the graft weakens during ligamentization (in the case of reconstruction).

Conclusion

While the diversity and availability of new biological technologies in orthopedic sports medicine surgery continues to increase, the literature remains inconclusive regarding the optimal indications for their implementation. The results of long-term follow-up have led to increasing recognition of the limitations in the surgical treatment of common athletic injuries like ACL tears, and bioaugmentation may offer some solutions in this regard. Nevertheless, bioaugmentation must not be regarded as a panacea in this regard. The high level of public awareness surrounding biological treatments related to their use by professional athletes may also be contributing to unreasonable expectations regarding the regenerative capacity of these interventions.[151] As with many interventions, bioaugmentation strategies seem to show the most promise when implemented with a targeted approach, in order to address specific biological problems. The surgeon must also maintain realistic expectations regarding the capability of these technologies, and not lose sight of additional factors that may contribute to adverse outcomes in some patients. For example, bioaugmentation will never overcome problems with extremity alignment, which should instead be addressed through osteotomies.

Increasingly, the results of clinical work utilizing bioaugmentation with ACL repair and reconstruction provide valuable information about the ways in which the four principles of tissue engineering (cells, growth factors, scaffolds, and mechanical stimuli) can be combined into targeted interventions to overcome specific biological challenges. While much of the current clinical work in this field has employed one or two of the core tissue engineering principles, the next generation of bioaugmentation strategies will increasingly combine elements of all four. More research will be required to further elucidate which of these approaches show the most promise and greatest therapeutic advantage.

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