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. ACL reconstruction (ACLR) has traditionally been recommended as protective against subsequent meniscal injury and cartilage damage, and ultimately osteoarthritis. In contrast with a greater than 90% success rate and 67% good or excellent outcomes, more recent publications have found a higher rate of revision following ACLR, ranging from 10% to 15%, and similar rates of radiographic osteoarthritis as with nonoperative management at long-term follow-up. This has inspired many to search for opportunities for improvement in the surgical management of these common athletic injuries.
Historically considered as an unreliable treatment option associated with high failure rates and complications related to intra-articular immunogenic reactions, some authors have again begun to explore ACL repair for certain patients. Still others have sought ways to enhance the gold-standard of reconstruction. 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 more than the original grafted tendon–bone interface.

Following ACLR, the grafted tendon continues to mature and integrate via progressive cellular phases—acute inflammatory, revascularization, recellularization, and tissue remodeling phases, respectively. 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. This begins with neovascularization, followed by fibroblast repopulation. 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. Disorganized collagen fibrils dominate earlier in the process, but these too become longitudinally organized with maturation. When newly formed connective tissue predominates but has yet to undergo longitudinal reorganization, the graft is mechanically weak and prone to failure.

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. 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. This creates a relative weak point that can contribute to rerupture.

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. 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. 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. These multipotent tissue-adherent cells have the ability to differentiate into osteogenic, adipogenic, and chondrogenic lineages.

MSCs have also been shown to have fibroblastic capacity, and may therefore also have a role in tendon and ligament healing. Yet, the FDA restrictions on ex vivo expansion also apply to MSCs. 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.

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. 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. 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., which sought to answer whether an increasing platelet concentration in an ECM scaffold would improve graft biomechanical properties and/or...
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. Growth factors have short half-lives and diffuse slowly through the ECM to act locally. Cell proliferation, ECM synthesis, and/or matrix production Table 1. In particular, TGFβ may help prevent graft deterioration and enhance osseous ingrowth at the tunnel wall.

Angiogenesis and osteogenesis are integral to tendon–bone healing following ACLR. If perfusion is delayed following a reconstruction, the grafted tendon may degenerate. If tendon–bone healing is suboptimal, biomechanical strength of the grafted tendon may be sacrificed. 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. In animal studies, VEGF was shown to promote angiogenesis in the grafted tendon following ACLR. VEGF has been shown to exhibit a synergistic effect on tendon healing in concert with TGFβ. In a study assessing ACL healing, VEGF was found to promote angiogenesis that aided in the healing process.

BMP2 has been shown to induce MSC proliferation, osteogenic differentiation, chondrogenic differentiation, as well as collagen production. BMP2 has demonstrated beneficial effects in fracture healing in multiple studies. 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. Despite the enthusiasm surrounding these findings, these discoveries have proven difficult to implement in a clinically meaningful way.

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/
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. Bioocompatibility 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.50 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

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

| Material       | Product     | Manufacturer   | Structural | Bioinductive | Results                                                                 |
|----------------|-------------|----------------|------------|--------------|-------------------------------------------------------------------------|
| Biologically derived |             |                |            |              |                                                                         |
| Human dermis   | GraftJacket | Wright Medical | ++ +       | +            | No ACL-specific results or outcomes                                      |
| ECM            |             |                |            |              | 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                |
| 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 |
|                |             |                |            |              | 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 |
| Collagen       |             |                |            |              |                                                                         |
|                |             |                |            |              |                                                                         |
|                |             |                |            |              |                                                                         |
| 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-l-lactic acid | Poly-Tape | Yufu Itonaga   | ++          | −            | Limited relevant clinical or preclinical data available                |
|                |             |                |            |              | 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 |
|                |             |                |            |              | 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.
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. 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. Accordingly, multiple collagen-based products have been examined as scaffolds to enhance mechanical stability. While collagen has the advantage of acting as a biocompatible scaffold, several studies have demonstrated a lack of mechanical strength beyond 6 weeks. 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. In contrast, hyaluronic acid lacks the mechanical properties of collagen- and silk-based products, but is a biocompatible component of the ECM. 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. Alginate is another biocompatible polysaccharide that has the ability of encapsulating cells. It, too, lacks mechanical strength. 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.

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. Unfortunately, the mechanical strength achieved with these techniques remain less than ideal. 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.

**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. 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. The exact timing, strength, and direction of mechanical stimuli required for optimal cellular response is the subject of ongoing research. 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. Studies have shown that cells respond to mechanical stimuli by initiating integrin-mediated focal adhesions and cytoskeleton deformation.

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. 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. 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 neo-vascularization, 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 and collagen-based matrices. Interestingly, the effectiveness of collagen scaffolds appears to be enhanced by the presence of platelets. 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. 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.

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. 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. 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. Numerous animal studies have reported success with the use of amniotic tissue in tendon repair. Several clinical studies have reported preliminary results for extra-articular applications, including tendon repair in foot and ankle procedures. 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. 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. 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).

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. However, relatively high rates of complications have been reported with DIS alone. Evangelopoulos et al. 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). In a preclinical laboratory study, Ganntenbein et al. 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. 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, Ruprecht 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. Vadala et al.130 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 Komzak 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 plasmam-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.136 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 Bishop140 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
recently been increased attention on suture augmentation or internal bracing in the repair and reconstruction of many ligaments. Suture tape augmentation has also been used in the setting of ACL repair and reconstruction and even as a method of revising reconstructions. 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. 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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References

1. Kiapour AM and Murray MM. Basic science of anterior cruciate ligament injury and repair. Bone Joint Res 2014; 3(2): 20–31.
2. Willadsen EM, Zahn AB and Durall CJ. What is the most effective training approach for preventing noncontact ACL injuries in high school-aged female athletes? J Sport Rehabil 2019; 28: 94–98.
3. Lyman S, Koulouvaris P, Sherman S, et al. Epidemiology of anterior cruciate ligament reconstruction: trends, readmissions, and subsequent knee surgery. J Bone Joint Surg Am 2009; 91(10): 2321–2328.
4. Anderson CN and Anderson AF. Management of the anterior cruciate ligament–injured knee in the skeletally immature athlete. *Clin Sports Med* 2017; 36(1): 35–52.
5. Website. https://doi.org/10.1016/j.csm.2016.08.003 (accessed 22 September 2019).
6. Spindler KP and Wright RW. Clinical practice: anterior cruciate ligament tear. *N Engl J Med* 2008; 359(20): 2135–2142.
7. Bach BR Jr. ACL reconstruction: revisited, revised, reviewed. *J Knee Surg* 2004; 17(3): 125–126.
8. Carson EW, Anisko EM, Restrepo C, et al. Revision anterior cruciate ligament reconstruction: etiology of failures and clinical results. *J Knee Surg* 2004; 17(3): 127–132.
9. Samitier G, Marcano AI, Alentorn-Geli E, et al. Failure of anterior cruciate ligament reconstruction. *Arch Bone Jt Surg* 2015; 3: 220–240.
10. Van Yperen DT, Reijman M, Van Es EM, et al. Twenty-year follow-up study comparing operative versus nonoperative treatment of anterior cruciate ligament ruptures in high-level athletes. *Am J Sports Med* 2018; 46: 1129–1136.
11. O’Donoghue DH, Frank GR, Jeter GL, et al. Repair and reconstruction of the anterior cruciate ligament in dogs: factors influencing long-term results. *J Bone Joint Surg Am* 1971; 53(4): 710–718.
12. Murray MM, Flutie BM, Kalish LA, et al. The bridge-enhanced anterior cruciate ligament repair (BEAR) procedure: an early feasibility cohort study. *Orthop J Sports Med* 2016; 4(11): 2325967116672176.
13. Van der List JP, Vermeijden HD, Siervelt IN, et al. Arthroscopic primary repair of proximal anterior cruciate ligament tears seems safe but higher level of evidence is needed: a systematic review and meta-analysis of recent literature. *Knee Surg Sports Traumatol Arthrosc*. Epub Ahead of Print 5 September 2019. DOI: 10.1007/s00167-019-05697-8.
14. Evangelopoulos DS, Kohl S, Schwienbacher S, et al. Collagen application reduces complication rates of mid-substance ACL tears treated with dynamic intraligamentary stabilization. *Knee Surg Sports Traumatol Arthrosc* 2017; 25(8): 2414–2419.
15. Del Torto M, Enea D, Panfoli N, et al. Hamstrings anterior cruciate ligament reconstruction with and without platelet rich fibrin matrix. *Knee Surg Sports Traumatol Arthrosc* 2015; 23(6): 3614–3622.
16. Vadala A, Iorio R, De Carli A, et al. Platelet-rich plasma: does it help reduce tunnel widening after ACL reconstruction? *Knee Surg Sports Traumatol Arthrosc* 2013; 21(4): 824–829.
17. Silva A, Sampaoio R, Fernandes R, et al. Is there a role for adult non-cultivated bone marrow stem cells in ACL reconstruction? *Knee Surg Sports Traumatol Arthrosc* 2014; 22(1): 66–71.
18. Darabos N, Haspl M, Moser C, et al. Intraarticular application of autologous conditioned serum (ACS) reduces bone tunnel widening after ACL reconstructive surgery in a randomized controlled trial. *Knee Surg Sports Traumatol Arthrosc* 2011; 19: 36–46.
19. Takayama K and Kuroda R. Biological augmentation of anterior cruciate ligament grafts. *Oper Techn Orthop* 2017; 27: 33–37.
20. Sun L, Wu B, Tian M, et al. Comparison of graft healing in anterior cruciate ligament reconstruction with and without a preserved remnant in rabbits. *Knee* 2013; 20(6): 537–544.
21. Van Eck CF, Schkrhowsky JG, Working ZM, et al. Prospective analysis of failure rate and predictors of failure after anatomic anterior cruciate ligament reconstruction with allograft. *Am J Sports Med* 2012; 40(4): 800–807.
22. Abe S, Kurosaka M, Iguchi T, et al. Light and electron microscopic study of remodeling and maturation process in autogenous graft for anterior cruciate ligament reconstruction. *Arthroscopy* 1993; 9(4): 394–405.
23. Falconiero RP, DiStefano VJ and Cook TM. Revascularization and ligamentization of autogenous anterior cruciate ligament grafts in humans. *Arthroscopy* 1998; 14(2): 197–205.
24. Rougaff B, Shellbourne KD, Gerth PK, et al. Arthroscopic and histologic analysis of human patellar tendon autografts used for anterior cruciate ligament reconstruction. *Am J Sports Med* 1993; 21(2): 277–284.
25. Sanchez M, Amitua E, Azofra J, et al. Ligamentization of tendon grafts treated with an endogenous preparation rich in growth factors: gross morphology and histology. *Arthroscopy* 2010; 26(4): 470–480.
26. Claes S, Verdonk P, Forsyth R, et al. The “ligamentization” process in anterior cruciate ligament reconstruction: what happens to the human graft? A systematic review of the literature. *Am J Sports Med* 2011; 39(11): 2476–2483.
27. LaPrade RF, Geeslin AG, Murray IR, et al. Biologic treatments for sports injuries II think tank-current concepts, future research, and barriers to advancement, part 1: biologics overview, ligament injury, tendinopathy. *Am J Sports Med* 2016; 44(12): 3270–3283.
28. Hexter AT, Thangarajah T, Blunn G, et al. Biological augmentation of graft healing in anterior cruciate ligament reconstruction: a systematic review. *Bone Joint J* 2018; 100-B(3): 271–284.
29. Brophy RH, Kovacevic D, Imhauser CW, et al. Effect of short-duration low-magnitude cyclic loading versus immobilization on tendon-bone healing after ACL reconstruction in a rat model. *J Bone Joint Surg Am* 2011; 93(4): 381–393.
30. Cooper RR and Misol S. Tendon and ligament insertion: a light and electron microscopic study. *J Bone Joint Surg Am* 1970; 52(1): 1–20.
31. Petersen W and Laprell H. Insertion of autologous tendon grafts to the bone: a histological and immunohistochemical study of hamstring and patellar tendon grafts. *Knee Surg Sports Traumatol Arthrosc* 2000; 8(1): 26–31.
32. Gulotta LV, Kovacevic D, Ying L, et al. Augmentation of tendon-to-bone healing with a magnesium-based bone adhesive. *Am J Sports Med* 2008; 36(7): 1290–1297.
33. Ishibashi Y, Toh S, Okamura Y, et al. Graft incorporation within the tibial bone tunnel after anterior cruciate ligament reconstruction with bone-patellar tendon-bone autograft. *Am J Sports Med* 2001; 29(4): 473–479.
34. Matsumoto T, Ingham SM, Mifune Y, et al. Isolation and characterization of human anterior cruciate ligament-derived vascular stem cells. *Stem Cells Dev* 2012; 21(6): 859–872.
35. Human cells, tissues, cellular and tissue-based products, https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcrf/ CFRSearch.cfm?fr=1271.3 (accessed 3 October 2019).
36. Van Eijk F, Saris DB, Riesle J, et al. Tissue engineering of ligaments: a comparison of bone marrow stromal cells, anterior cruciate ligament, and skin fibroblasts as cell source. *Tissue Eng* 2004; 10(5–6): 893–903.
37. Chen J, Altman GH, Karageorgiou V, et al. Human bone marrow stromal cell and ligament fibroblast responses on RGD-modified silk fibers. *J Biomed Mater Res A* 2003; 67(2): 559–570.

38. Dominici M, Nichols K, Srivastava A, et al. Positioning a scientific community on unproven cellular therapies: the 2015 international society for cellular therapy perspective. *Cytotherapy* 2015; 17(12): 1663–1666.

39. Lui PPY, Rui YF, Ni M, et al. Tenogenic differentiation of stem cells for tendon repair—what is the current evidence? *J Tissue Eng Regen Med* 2011; 5(8): e144–e163.

40. Leong NL, Petrigliano FA and McAllister DR. Current tissue engineering strategies in anterior cruciate ligament reconstruction. *J Biomed Mater Res A* 2014; 102: 1614–1624.

41. Gantenbein B, Gadhari N, Chan SC, et al. Mesenchymal stem cells and collagen patches for anterior cruciate ligament repair. *World J Stem Cells* 2015; 7(2): 521–534.

42. Chu CR, Rodeo S, Bhuiani N, et al. Optimizing clinical use of biologics in orthopaedic surgery: consensus recommendations from the 2018 AAOS/NIH U-13 conference. *J Am Acad Orthop Surg* 2019; 27(2): e50–e63.

43. Mahapatra P, Horriat S and Anand BS. Anterior cruciate ligament repair—past, present and future. *J Exp Orthop* 2018; 5(1): 20.

44. Pizzuti NS, Hussain ZB, Chahlaj J, et al. Variability in the preparation, reporting, and use of bone marrow aspirate concentrate in musculoskeletal disorders: a systematic review of the clinical orthopaedic literature. *J Bone Joint Surg Am* 2018; 100(6): 517–525.

45. Turner L and Knoopfner P. Selling stem cells in the USA: assessing the direct-to-consumer industry. *Cell Stem Cell* 2016; 19(2): 154–157.

46. Di Matteo B, Filardo G, Kon E, et al. Platelet-rich plasma: evidence for the treatment of patellar and Achilles tendinopathy—a systematic review. *Musculoskelet Surg* 2015; 99: 1–9.

47. Kon E, Filardo G, Di Matteo B, et al. PRP for the treatment of cartilage pathology. *Open Orthop J* 2013; 7: 120–128.

48. Filardo G, Kon E, Roffi A, et al. Platelet-rich plasma: why intra-articular? A systematic review of preclinical studies and clinical evidence on PRP for joint degeneration. *Knee Surg Sports Traumatol Arthrosc* 2015; 23(9): 2459–2474.

49. Andriolo L, Di Matteo B, Kon E, et al. PRP augmentation for ACL reconstruction. *Biomedi Res Int* 2015; 2015: 371746.

50. Fleming BC, Proffen BL, Vavken P, et al. Increased platelet concentration does not improve functional graft healing in bio-enhanced ACL reconstruction. *Knee Surg Sports Traumatol Arthrosc* 2015; 23: 1161–1170.

51. Khambete H, Keservani RK, Kesharwani RK, et al. Emerging trends of nanobiomaterials in hard tissue engineering. *Nanobiomater Hard Tissue Eng* 2016; 4: 63–101.

52. Lamalice L, Le Boeuf F and Huot J. Endothelial cell migration during angiogenesis. *Circ Res* 2007; 100: 782–794.

53. Thomopoulous S, Das R, Sakiyama-Elbert S, et al. bFGF and PDGF-BB for tendon repair: controlled release and biologic activity by tendon fibroblasts in vitro. *Ann Biomed Eng* 2010; 38(2): 225–234.

54. Hamada Y, Katoh S, Hibino N, et al. Effects of monofilament nylon coated with basic fibroblast growth factor on endogenous intrasynovial flexor tendon healing. *J Hand Surg Am* 2006; 31(4): 530–540.

55. Schmidt CC, Georgescu HI, Kwok CK, et al. Effect of growth factors on the proliferation of fibroblasts from the medial collateral and anterior cruciate ligaments. *J Orthop Res* 1995; 13(2): 184–190.

56. Scherping SC Jr, Schmidt CC, Georgescu HI, et al. Effect of growth factors on the proliferation of ligament fibroblasts from skeletally mature rabbits. *Connect Tissue Res* 1997; 36(1): 1–8.

57. Haddad-Weber M, Prager P, Kunz M, et al. BMP12 and BMP13 gene transfer induce ligamentogenic differentiation in mesenchymal progenitor and anterior cruciate ligament cells. *Cytotherapy* 2010; 12(4): 505–513.

58. Yasuda K, Tomita F, Yamazaki S, et al. The effect of growth factors on biomechanical properties of the bone-patellar tendon-bone graft after anterior cruciate ligament reconstruction: a canine model study. *Am J Sports Med* 2004; 32(4): 870–880.

59. Vavken P, Sadoghi P and Murray MM. The effect of platelet-concentrates on graft maturation and graft-bone interface healing in anterior cruciate ligament reconstruction in human patients: a systematic review of controlled trials. *Arthroscopy* 2011; 27(11): 1573–1583.

60. Vavken P, Fleming BC, Mastrangelo AN, et al. Biomechanical outcomes after bioenhanced anterior cruciate ligament repair and anterior cruciate ligament reconstruction are equal in a porcine model. *Arthroscopy* 2012; 28(5): 672–680.

61. Goradia VK, Rochat MC, Kida M, et al. Natural history of a hamstring tendon autograft used for anterior cruciate ligament reconstruction in a sheep model. *Am J Sports Med* 2000; 28(1): 40–46.

62. Ju Y-J, Tohyama H, Kondo E, et al. Effects of local administration of vascular endothelial growth factor on properties of the in situ frozen-thawed anterior cruciate ligament in rabbits. *Am J Sports Med* 2006; 34(1): 84–91.

63. Wei X, Mao Z, Hou Y, et al. Local administration of TGFβ1/VEGF165 gene-transduced bone mesenchymal stem cells for Achilles allograft replacement of the anterior cruciate ligament in rabbits. *Biochem Biophys Res Commun* 2011; 406: 204–210.

64. Takayama K, Kawakami Y, Mifune Y, et al. The effect of blocking angiogenesis on anterior cruciate ligament healing following stem cell transplantation. *Biomaterials* 2015; 60: 9–19.

65. Abate M, Gravare Silbernagel K, Siljeholm C, et al. Pathogenesis of tendinopathies: inflammation or degeneration? *Arthritis Res Ther* 2009; 11: 235.

66. Adams MK, Goodrich LR, Rao S, et al. Equine bone marrow-derived mesenchymal stromal cells (BMDMSCs) from the ilium and sternum: are there differences. *Arthritis Res Ther* 2013; 45(3): 372–375.

67. Ajuied A, Wong F, Smith C, et al. Anterior cruciate ligament injury and radiologic progression of knee osteoarthritis: a systematic review and meta-analysis. *Am J Sports Med* 2014; 42(9): 2242–2252.

68. Akita S, Akino K, Tanaka K, et al. A basic fibroblast growth factor improves lower extremity wound healing with a porcine-derived skin substitute. *J Trauma* 2008; 64(3): 809–815.

69. Friedlaender GE, Perry CR, Cole JD, et al. Osteogenic protein-1 (bone morphogenetic protein-7) in the treatment of
tibial nonunions. J Bone Joint Surg Am 2001; 83-A(Pt 2): S151–S118.
70. Govender S, Csimma C, Genant HK, et al. Recombinant human bone morphogenetic protein-2 for treatment of open tibial fractures: a prospective, controlled, randomized study of four hundred and fifty patients. J Bone Joint Surg Am 2002; 84(12): 2123–2134.
71. Anderson K, Seneviratne AM, Izawa K, et al. Augmentation of tendon healing in an intraarticular bone tunnel with use of a bone growth factor. Am J Sports Med 2001; 29(6): 689–698.
72. Lee K, Silva EA and Mooney DJ. Growth factor delivery-based tissue engineering: general approaches and a review of recent developments. J R Soc Interface 2011; 8(55): 153–170.
73. Hutmacher DW. Scaffolds in tissue engineering bone and cartilage. Biomaterials 2000; 21: 2529–2543.
74. Barber FA, McGarry JE, Herbert MA, et al. A biomechanical study of Achilles tendon repair augmentation using GraftJacket matrix. Foot Ankle Int 2008; 29(3): 329–333.
75. Bond JL, Dopirak RM, Higgins J, et al. Arthroscopic replacement of massive, irreparable rotator cuff tears using a Graftofoat allograft: technique and preliminary results. Arthroscopy 2008; 24(4): 403–409.
76. Song L, Olsen RE, Spalazzi JP, et al. Biomechanical evaluation of acellular collagen matrix augmented Achilles tendon repair in sheep. J Foot Ankle Surg 2010; 49(5): 438–441.
77. Badhe SP, Lawrence TM, Smith FD, et al. An assessment of porcine dermal xenograft as an augmentation graft in the treatment of extensive rotator cuff tears. J Shoulder Elbow Surg 2008; 17(Suppl. 1): 35S–39S.
78. Schlegel TF, Abrams JS, Bushnell BD, et al. Radiologic and clinical evaluation of a bioabsorbable collagen implant to treat partial-thickness tears: a prospective multicenter study. J Shoulder Elbow Surg 2018; 27(2): 242–251.
79. Altman GH, Horan RL, Weitzel P, et al. Clinical, mechanical and histopathological evaluation of a bioengineered long-term biodegradable silk fibroin graft in a one year goat study for development of a functional autologous anterior cruciate ligament. Orthop Proc 2018; 94: 25.
80. Altman GH, Horan RL, Lu HH, et al. Silk matrix for tissue engineered anterior cruciate ligaments. Biomaterials 2002; 23(20): 4131–4141.
81. Tanaka N, Sakahashi H, Hirose K, et al. Augmented subscapularis muscle transposition for rotator cuff repair during shoulder arthroplasty in patients with rheumatoid arthritis. J Shoulder Elbow Surg 2006; 15(1): 2–6.
82. Koh JL, Szomor Z, Murrell GAC, et al. Supplementation of rotator cuff repair with a bioresorbable scaffold. Am J Sports Med 2002; 30(3): 410–413.
83. Gersoff WK, Boynskii CC, Cook CR, et al. Evaluation of a novel degradable synthetic biomaterial patch for augmentation of tendon healing in a large animal model. J Knee Surg 2019; 32(5): 434–440.
84. Robinson PM and Muir LT. Foreign body reaction associated with Artelon: report of three cases. J Hand Surg Am 2011; 36(1): 116–120.
85. Petriccioleti D, Bertone C, Marchi G, et al. Open repair of isolated traumatic subscapularis tendon tears with a synthetic soft tissue reinforcement. Musculoskelet Surg 2013; 97(Suppl. 1): 63–68.
86. Longo UG, Lamberti A, Maffulli N, et al. Tendon augmentation grafts: a systematic review. Br Med Bull 2010; 94: 165–188.
87. Cole BJ, Gomoll AH, Yanke A, et al. Biocompatibility of a polymer patch for rotator cuff repair. Knee Surg Sports Traumatol Arthrosc 2007; 15(5): 632–637.
88. Dyment NA, Kazemi N, Aschbacher-Smith LE, et al. The relationships among spatiotemporal collagen gene expression, histology, and biomechanics following full-length injury in the murine patellar tendon. J Orthop Res 2012; 30(1): 28–36.
89. Sharma P and Maffulli N. Basic biology of tendon injury and healing. Surgeon 2005; 3(5): 309–316.
90. Caruso AB and Dunn MG. Changes in mechanical properties and cellularity during long-term culture of collagen fiber ACL reconstruction scaffolds. J Biomed Mater Res A 2005; 73(4): 388–397.
91. Dunn MG, Liesh JB, Tiku ML, et al. Development of fibroblast-seeded ligament analogs for ACL reconstruction. J Biomed Mater Res 1995; 29(11): 1363–1371.
92. Bellincampi LD, Closkey RF, Prasad R, et al. Viability of fibroblast-seeded ligament analogs after autogenous implantation. J Orthop Res 1998; 16(4): 414–420.
93. Goulet F, Rancourt D, Cloutier R, et al. Tendons and ligaments. In: Lanza R, Langer R and Vacanti J (eds) Principles of tissue engineering. Cambridge, MA: Academic Press, 2000, pp. 711–722.
94. Shao H-J, Chen CS, Lee Y-T, et al. The phenotypic responses of human anterior cruciate ligament cells cultured on poly(e-caprolactone) and chitosan. J Biomed Mater Res A 2009; 93A(4): 1297–1305.
95. Iwasaki N, Yamane S-T, Majima T, et al. Feasibility of polysaccharide hybrid materials for scaffolds in cartilage tissue engineering: evaluation of chondrocyte adhesion to poly(ε-caprolactone) and chitosan. Biomaterials 2004; 5(3): 828–833.
96. Lu HH, Cooper JA Jr, Manuel S, et al. Anterior cruciate ligament regeneration using braided biodegradable scaffolds: in vitro optimization studies. Biomaterials 2005; 26(23): 4805–4816.
97. Walters VI, Kwansa AL and Freeman JW. Design and analysis of braided-twill collagen scaffolds. Connect Tissue Res 2012; 53(3): 255–266.
98. Koob TJ, Willis TA, Qiu YS, et al. Biocompatibility of NDGA-polymerized collagen fibers. II. Attachment, proliferation, and migration of tendon fibroblasts in vitro. J Biomed Mater Res 2001; 56(1): 40–48.
99. Panas-Perez E, Gatt CJ and Dunn MG. Development of a silk and collagen fiber scaffold for anterior cruciate ligament reconstruction. J Mater Sci Mater Med 2013; 24(1): 257–265.
100. Woo SL-Y, Gomez MA, Woo Y-K, et al. Mechanical properties of tendons and ligaments. Bioengineering 1982; 19: 397–408.
101. Mody BS, Howard L, Harding ML, et al. The ABC carbon and polyester prosthetic ligament for ACL-deficient knees: early results in 31 cases. J Bone Joint Surg Br 1993; 75(5): 818–821.
102. Moreau JE, Bramono DS, Horan RL, et al. Sequential biochemical and mechanical stimulation in the development of...
tissue-engineered ligaments. *Tissue Eng Part A* 2008; 14(7): 1161–1172.

103. Henshaw DR, Attia E, Bhargava M, et al. Canine ACL fibroblast integrin expression and cell alignment in response to cyclic tensile strain in three-dimensional collagen gels. *J Orthop Res* 2006; 24(3): 481–490.

104. Berry CC, Cacou C, Lee DA, et al. Dermal fibroblasts respond to mechanical conditioning in a strain profile dependent manner. *Biorheology* 2003; 40(1–3): 337–345.

105. Berry SM and Green MH. Hyaluronan: a potential carrier for growth factors for the healing of ligamentous tissues. *Wound Repair Regen* 1997; 5(1): 33–38.

106. Robayo LM, Moulin VJ, Tremblay P, et al. New ligament healing model based on tissue-engineered collagen scaffolds. *Wound Repair Regen* 2011; 19(1): 38–48.

107. Murray MM, Spindler KP, Devin C, et al. Use of a collagen-platelet rich plasma scaffold to stimulate healing of a central defect in the canine ACL. *J Orthop Res* 2006; 24(4): 820–830.

108. Joshi SM, Mastrangelo AN, Magarian EM, et al. Collagen-platelet composite enhances biomechanical and histologic healing of the porcine anterior cruciate ligament. *Am J Sports Med* 2009; 37(12): 2401–2410.

109. Fleming BC, Magarian EM, Harrison SL, et al. Collagen scaffold supplementation does not improve the functional properties of the repaired anterior cruciate ligament. *J Orthop Res* 2014; 32(2): 291–295.

110. Murray MM, Spindler KP, Abreu E, et al. Collagen-platelet rich plasma hydrogel enhances primary repair of the porcine anterior cruciate ligament. *J Orthop Res* 2007; 25(1): 81–91.

111. Yoshida R, Cheng M and Murray MM. Increasing platelet concentration in platelet-rich plasma inhibits anterior cruciate ligament cell function in three-dimensional culture. *J Orthop Res* 2014; 32(2): 291–295.

112. Figueroa D, Espinosa M, Calvo R, et al. Anterior cruciate ligament regeneration using mesenchymal stem cells and collagen type I scaffold in a rabbit model. *Knee Surg Sports Traumatol Arthrosc* 2014; 22(5): 1196–1202.

113. Jay RM. Initial clinical experience with the use of human amniotic membrane tissue during repair of posterior tibial and Achilles tendons. AF Cell Medical, 2009, https://www.aazisk.com/wp-content/uploads/2018/03/Jay-Initial-Clinical-Experience-with-the-Use-of-Human-Amniotic-Membrane-Tissue-During-Repair-Of-Posterior-Tibial-and-Achilles-Tendons-MfMedx-OEM-AFcell.pdf

114. Saw VPJ, Minassian D, Dart JKG, et al. Amniotic membrane transplantation for ocular disease: a review of the first 233 cases from the UK user group. *Br J Ophthalmol* 2007; 91(8): 1042–1047.

115. Lei J, Priddy LB, Lim JJ, et al. Identification of extracellular matrix components and biological factors in micromized dehydrated human amnion/chorion membrane. *Adv Wound Care* 2017; 6(2): 43–53.

116. Heckmann N, Auran R and Mirzayan R. Application of amniotic tissue in orthopedic surgery. *Am J Orthop* 2016; 45(7): E421–E425.

117. Riboh JC, Saltzman BM, Yanke AB, et al. Human amniotic membrane–derived products in sports medicine: basic science, early results, and potential clinical applications. *Am J Sports Med* 2016; 44(9): 2425–2434.

118. Zelen CM, Poka A and Andrews J. Prospective, randomized, blinded, comparative study of injectable micronized dehydrated amniotic/chorionic membrane allograft for plantar fasciitis—a feasibility study. *Foot Ankle Int* 2013; 34(10): 1332–1339.

119. Hanselman AE, Tidwell JE and Santrock RD. Cryopreserved human amniotic membrane injection for plantar fasciitis: a randomized, controlled, double-blind pilot study. *Foot Ankle Int* 2015; 36(2): 151–158.

120. Murray MM, Kalish L, Fleming BC, et al. Bridge-enhanced ACL repair: two year results of the first in human study. *Orthop J Sports Med* 2019; 7: 2352967118824356.

121. Eggli S, Kohlhof H, Zumstein M, et al. Dynamic intraligamentary stabilization: novel technique for preserving the ruptured ACL. *Knee Surg Sports Traumatol Arthrosc* 2015; 23(4): 1215–1221.

122. Henle P, Roder C, Perler G, et al. Dynamic intraligamentary stabilization (DIS) for treatment of acute anterior cruciate ligament ruptures: case series experience of the first three years. *BMJ Musculoskeletal Disord* 2015; 16: 27.

123. Osti M, El Attal R, Doskar W, et al. High complication rate following dynamic intraligamentary stabilization for primary repair of the anterior cruciate ligament. *Knee Surg Sports Traumatol Arthrosc* 2019; 27: 29–36.

124. Ahmad SS, Schreiner AJ, Hirschmann MT, et al. Dynamic intraligamentary stabilization for ACL repair: a systematic review. *Knee Surg Sports Traumatol Arthrosc* 2019; 27(3): 13–20.

125. Radice F, Yanez R, Gutierrez V, et al. Comparison of magnetic resonance imaging findings in anterior cruciate ligament grafts with and without autologous platelet-derived growth factors. *Arthroscopy* 2010; 26(1): 50–57.

126. Rupreht M, Jevtic V, Sersa I, et al. Evaluation of the tibial tunnel after intraoperatively administered platelet-rich plasma gel during anterior cruciate ligament reconstruction using diffusion weighted and dynamic contrast-enhanced MRI. *J Magn Reson Imaging* 2013; 37(4): 928–935.

127. Vogrin M, Rupreht M, Dinevski D, et al. Effects of a platelet gel on early graft revascularization after anterior cruciate ligament reconstruction: a prospective, randomized, double-blind, clinical trial. *Eur Surg Res* 2010; 45(2): 77–85.

128. Seijas R, Ares O, Catala J, et al. Magnetic resonance imaging evaluation of patellar tendon graft remodelling after anterior cruciate ligament reconstruction with or without platelet-rich plasma. *J Orthop Surg* 2013; 21: 10–14.

129. Orrego M, Larrain C, Rosales J, et al. Effects of platelet concentrate and a bone plug on the healing of hamstring tendons in a bone tunnel. *Arthroscopy* 2008; 24(12): 1373–1380.

130. Mizratolooei F, Alamdari MT and Khalkhali HR. The impact of platelet-rich plasma on the prevention of tunnel widening in anterior cruciate ligament reconstruction using quadrupled autologous hamstring tendon. *Bone Joint J* 2013; 95-B(1): 65–69.

131. Valenti Azcarate A, Lamo-Espinosa J, Aquerreta Beola JD, et al. Comparison between two different platelet-rich plasma preparations and control applied during anterior cruciate ligament reconstruction: is there any evidence to support their use? *Injury* 2014; 45(Suppl. 4): S36–S41.

132. Komzák M, Hart R, Smid P, et al. The effect of platelet-rich plasma on graft healing in reconstruction of the anterior...
cruciate ligament of the knee joint: prospective study. Acta Chir Orthop Traumatol Cech 2015; 82(2): 135–139.

133. Iorio R, Di Sanzo V, Vadala A, et al. Nanohydroxyapatite-based bone graft substitute in tunnel enlargement after ACL surgery: RMN study. Clin Ter 2013; 164(2): e101–e116.

134. Mutsuzaki H, Kanamori A, Ikeda K, et al. Effect of calcium phosphate-hybridized tendon graft in anterior cruciate ligament reconstruction: a randomized controlled trial. Am J Sports Med 2012; 40(8): 1772–1780.

135. Mutsuzaki H, Kinugasa T, Ikeda K, et al. Morphological changes in the femoral and tibial bone tunnels after anatomic single-bundle anterior cruciate ligament reconstruction using a calcium phosphate-hybridized tendon graft in 2 years of follow-up. Orthop Traumatol Surg Res 2019; 105(4): 653–660.

136. Kroon ME, Van Schie ML, Van der Vecht B, et al. Collagen type I retards tube formation by human microvascular endothelial cells in a fibrin matrix. Angiogenesis 2002; 5(4): 257–265.

137. Berdis AS, Veale K and Fleissner PR Jr. Outcomes of anterior cruciate ligament reconstruction using biologic augmentation in patients 21 years of age and younger. Arthroscopy 2019; 35(11): 3107–3113.

138. Wiggins AJ, Grandhi RK, Schneider DK, et al. Risk of secondary injury in younger athletes after anterior cruciate ligament reconstruction. Am J Sports Med 2016; 44(7): 1861–1876.

139. Woodall BM, Elena N, Gamboa JT, et al. Anterior cruciate ligament reconstruction with amnion biological augmentation. Arthrosc Tech 2018; 7(4): e355–e360.

140. Lavender C and Bishop C. The fertilized anterior cruciate ligament: an all-inside anterior cruciate ligament reconstruction augmented with amnion, bone marrow concentrate, and a suture tape. Arthrosc Tech 2019; 8(6): e555–e559.

141. Bio ACL reconstruction with amnion collagen matrix wrap and stem cells case series. ClinicalTrials.gov. https://clinicaltrials.gov/ct2/show/NCT03294720 (accessed 17 October 2019).

142. Bodendorfer BM, Anoushiravani AA, Feeley BT, et al. Anterior cruciate ligament bracing: evidence in providing stability and preventing injury or graft re-rupture. Phys Sportsmed 2013; 41: 92–102.

143. Bodendorfer BM, Arnold NR, Shu HT, et al. Do neoprene sleeves and prophylactic knee braces affect neuromuscular control and cutting agility? Phys Ther Sport 2019; 39: 23–31.

144. Bodendorfer BM, Looney AM, Lipkin SL, et al. Biomechanical comparison of ulnar collateral ligament reconstruction with the docking technique versus repair with internal bracing. Am J Sports Med 2018; 46(14): 3495–3501.

145. Ulku TK, Kocaoglu B, Tok O, et al. Arthroscopic suture-tape internal bracing is safe as arthroscopic modified Broström repair in the treatment of chronic ankle instability. Knee Surg Sports Traumatol Arthrosoc 2020; 28: 227–232.

146. McWilliam JR and Mackay G. The internal brace for mid-substance Achilles ruptures. Foot Ankle Int 2016; 37(7): 794–800.

147. Gagliardi AG, Carry PM, Parikh HB, et al. ACL repair with suture ligament augmentation is associated with a high failure rate among adolescent patients. Am J Sports Med 2019; 47(3): 560–566.

148. Heusdens CHW, Hopper GP, Dossche L, et al. Anterior cruciate ligament repair with independent suture tape reinforcement: a case series with 2-year follow-up. Knee Surg Traumatol Arthrosoc 2019; 27(1): 60–67.

149. Bodendorfer BM, Michaelson EM, Shu HT, et al. Suture augmented versus standard anterior cruciate ligament reconstruction: a matched comparative analysis. Arthroscopy 2019; 35(7): 2114–2122.

150. Shu HT, Day J, Bodendorfer BM, et al. Anterior cruciate ligament retensioning—a novel revision procedure using suture augmentation: a case report with a 3-year follow-up. JBJS Case Connect 2020; 10(2): e0092.

151. Schwarz A. New procedure uses athletes’ own blood to treat injuries. New York Times, 16 February 2009, p. A1.