

## **Fibrin-Based Biomaterial Systems to Enhance Anterior Cruciate Ligament Healing**

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**Abstract**

Anterior cruciate ligament (ACL) tears are a common and potentially career-ending injury, particularly for athletes and soldiers. Partial and complete ruptures of this ligament cause instability in the knee, and the ACL does not have the capacity for healing due, in part, to its position within the highly thrombolytic synovial fluid environment of the knee joint. Traditional methods of ACL reconstruction, such as graft replacement with attached bone anchors for bone integration, restore stability, but do not prevent the development of post-traumatic osteoarthritis. To enhance therapeutic treatment options, novel fibrin-based technologies and repair techniques have been recently explored and show promise for improved patient outcomes. Through modification of existing surgical methods, such as the use of fibrin glues incorporating growth factors and cells and the implementation of scaffolds containing platelet-rich plasma, platelet-rich fibrin, and other blood derivatives, surgeons are attempting to overcome the shortcomings of traditional treatments. This mini-review will detail current efforts using fibrin-based treatments and discuss opportunities to further enhance ACL healing.

## Introduction

The anterior cruciate ligament (ACL) is an essential soft tissue stabilizer within the knee joint, connecting the lateral femoral condyle to the tibial plateau. Like other ligaments and tendons, the ACL is primarily composed of a highly aligned, hierarchical, and fibrous collagen matrix (Hirokawa, Yamamoto, & Kawada, 2002; Stocchi et al., 1992; Zhu, Zhang, Ma, Zhou, & Ao, 2012). This structure allows the ACL to resist excessive translation and rotation of the knee joint during daily activities and sports (Bates, Schilaty, Nagelli, Krych, & Hewett, 2019; Woo, Abramowitch, Kilger, & Liang, 2006; Woo & Fisher, 2009). ACL injuries are debilitating, having an annual incidence of 200,000 in the United States alone (Griffin et al., 2006; Mall et al., 2014). Unlike extra-articular ligaments, intra-articular ligaments like the ACL have limited healing capacity, likely due to the surrounding synovial fluid environment (M. M. Murray, Martin, Martin, & Spector, 2000; Spindler, Murray, Devin, Nanney, & Davidson, 2006).

Various materials have been used in ACL reconstruction and repair (Fig. 1). The current clinical standard of care for many active patients is surgical intervention for knee stability restoration, namely ACL reconstruction using soft tissue grafts. Yet, 30-80% of patients develop osteoarthritis (OA) within 10-15 years post-injury (Barenius et al., 2014; Luc, Gribble, & Pietrosimone, 2014; Månsson, Sernert, Rostgard-Christensen, & Kartus, 2015; Øiestad, Engebretsen, Storheim, & Risberg, 2009; Whittaker, Woodhouse, Nettel-Aguirre, & Emery, 2015). These rates are similar to those of conservative non-surgical treatment (Calvo et al., 2015; Johnson, Roe, Salmon, Pinczewski, & Hunter, 2016) and the cost of each reconstruction surgery is ~\$5,000-\$50,000 (Brophy, Wright, & Matava, 2009; Nagda, Altobelli, Bowdry, Brewster, & Lombardo, 2010), raising the question of whether alternative treatments may be better for certain patient populations (Dallo et al., 2017). Synthetic and natural materials for ACL reconstruction

and repair have been reviewed elsewhere (Fig 1) (da Silveira Franciozi et al., 2014; Kiapour & Murray, 2014; Legnani, Ventura, Terzaghi, Borgo, & Albisetti, 2010; Nau & Teuschl, 2015; Taylor, Khair, Roberts, & DiFelice, 2015). Synthetic materials used in the context of ACL reconstruction include carbon fibers, polytetrafluorethylene (PTFE), polyester, polypropylene (PP), polyglycolic acid (PGA), polydioxanone (PDO), and poly L-lactic acid (PLLA) grafts. A number of natural materials have been used to stimulate ACL repair, including collagen, fibrin, extracellular matrix, alginate, chitosan, hyaluronic acid (HA), and silk. The goal of this review is to explore the use of fibrin-based approaches for promoting ACL healing.

The limitations of ACL reconstruction using traditional techniques and materials has led many to re-explore the capacity of the ACL to heal in animal models and humans (Fujimoto, Sumen, Ochi, & Ikuta, 2002; M. M. Murray et al., 2000; Ng, Oakes, McLean, Deacon, & Lampard, 1996; Steadman, Cameron-Donaldson, Briggs, & Rodkey, 2010). Techniques that take advantage of native coagulation have shown promise for treating ACL injury. This mini-review will provide an overview of recent advancements in fibrin-based approaches and highlight potential areas of future research. Although more work is needed, these promising approaches have the potential to alter ACL injury treatment paradigms by promoting restoration rather than replacement.

## **Overview of ACL healing**

Following injury, most extra-articular ligaments have the capacity to heal via a typical wound healing process, divided into the temporally overlapping phases of hemostasis, inflammation, proliferation, and remodeling (Frank et al., 1983; Leong et al., 2020; Woo et al., 2006). During the hemostasis and inflammatory phases which occur minutes after acute injury, platelets become activated, are recruited to the injury site, adhere to the injured tissue, and

aggregate to form a primary hemostatic plug. In secondary hemostasis, fibrinogen is crosslinked into the clot and cleaved by thrombin to form a fibrin clot; the fibrin network is later retracted by platelets, increasing mechanical stability (Hou et al., 2015; Tucker, Sage, & Gibbins, 2012). This provisional scaffold bridges the tissue together, allowing later phases of healing to occur (Fig 2A). Inflammation occurs immediately, and neutrophils and macrophages remove foreign material from the wound site. Days after injury, the proliferative phase begins, in which growth factors released by platelets further attract immune cells, stimulate angiogenesis, and recruit fibroblasts (Hauser, 2013). Fibroblasts migrate into the wound area, proliferate, and deposit a type III collagen extracellular matrix (ECM) (Vihersaari, Kivisaari, & Ninikoski, 1974). The remodeling phase begins several weeks post-injury and involves the replacement of type III collagen with type I collagen and improved ECM alignment (Yang, Rothrauff, & Tuan, 2013). Remodeling can proceed for several years. Despite this scar tissue being mechanically inferior to native ligament tissue, overall function is restored.

Unlike extra-articular ligaments, intra-articular ligaments such as the ACL have limited healing capability. One reason is the poor vascularity of the ACL, which severely limits the infiltration of cytokines, platelets, and other components that comprise the wound healing response. This prevents the formation of a provisional scaffold, further inhibiting the production and secretion of pro-healing cytokines and the recruitment of cells (Nyland et al., 2020). Indeed, it is suggested that the more highly vascularized proximal portion of the ACL has a greater healing potential than the less vascularized medial and distal portions (Nyland et al., 2020; Petersen & Tillmann, 1999).

A second reason for poor healing is the surrounding synovial fluid environment (M. M. Murray et al., 2000). Synovial fluid has lubricative properties, protecting the knee joint from

general wear-and-tear. As part of this function, synovial fluid contains enzymes, such as matrix metalloproteinase 1 (MMP-1), elastase, and plasmin, that are responsible for the degradation of collagen, elastin, and fibrin, respectively (Alain et al., 1992; Gysen, Malaise, Caspar, & Franchimont, 1985; Kleesiek et al., 1986; Palmer, Stanford, & Murray, 2011). During the normal wound healing process, plasmin, an activated zymogen triggered by tissue plasminogen activator (tPA), controls clot degradation by cleaving fibrinogen and fibrin and generating soluble fibrin degradation products (FDPs) (Cesarman-Maus & Hajjar, 2005). Because of the high concentration of plasmin in the synovial fluid (Fig 2B), any provisional scaffold formed despite poor vascular material transport is quickly degraded, preventing healing of the ACL (Gagliardi et al., 2019; Harrold, 1961). Concentrations of these proteolytic enzymes have been found to increase post-trauma, and this can impact ACL tear clotting and tissue repair (Palmer et al., 2011).

Thus, to promote ACL healing, methods are needed to overcome these biological challenges and restore the fundamental processes involved in ligament healing. Since fibrin is a critical component of hemostasis and provides preliminary scaffolding for tissue repair, fibrin-based approaches are a logical choice to enhance ACL healing. Some of the most common fibrin-based products used in ligament tear treatment are fibrin glues, platelet-rich plasma (PRP), and platelet-rich fibrin (PRF). Applications of these fibrin-rich products to ACL repair are discussed in subsequent sections.

## **Fibrin**

Fibrin glue has been used as a hemostatic agent for a variety of purposes, with some popular commercial formulations being Tisseel, Evicel, and Evarrest (Spotnitz, 2014). Fibrin forms a hydrogel and is created through the cleavage of fibrinopeptides A and B from fibrinogen via the

serine protease thrombin, which exposes the A and B knobs for binding with holes a and b of adjacent fibrin(ogen) molecules (Fig 3) (Brown & Barker, 2014). Nascent fibrin molecules polymerize further with other fibrin molecules to ultimately form a fibrillar matrix. The fibrin network is stabilized through crosslinking via Factor XIIIa. Platelet binding to fibrin via GPIIb/IIIa surface receptors also stabilizes fibrin networks by providing crosslinks and increasing fibrin density via platelet-mediated retraction (Nandi & Brown, 2016). Fibrin glues are typically administered via a double-barreled syringe containing fibrinogen and thrombin in separate compartments that are mixed directly at the application site; this creates a thick fibrin clot (Brown & Barker, 2014) within seconds that is slowly reabsorbed by the body over a time frame of days to weeks (Jackson, 2001; Radosevich, Goubran, & Burnouf, 1997). FDA-approved fibrin glue formulations have fibrinogen concentrations that are 20-30 times higher than in physiological conditions (Mintz et al., 2001; Tennent et al., 2007), suggesting that such approaches could be advantageous for use in the highly fibrinolytic environment of the ACL because these supraphysiological fibrinogen and thrombin concentrations result in clots resistant to fibrinolysis. However, high concentration fibrin glues can inhibit fibroblast migration and nutrient diffusion into fibrin clots due to decreased porosity of the final mesh (Fig 4) (Spotnitz, 2014; Vavken, Joshi, & Murray, 2011; Yeung, Faraj, McIntosh, Dhillon, & Dua, 2016). Furthermore, in rodent models (Kondo, Yasuda, Yamanaka, Minami, & Tohyama, 2005; X. Sun et al., 2017), fibrin sealant alone did not impact joint stability or tissue biomechanics relative to untreated controls, suggesting the limited impact of fibrin-only scaffolds (Kondo et al., 2005). Additionally, fibrin-based scaffolds exposed to MMP-1, elastase or plasmin *in vitro* degrade rapidly (Palmer et al., 2011), suggesting the need for enhanced clot stability to achieve healing (Martha M. Murray & Fleming, 2013).

As a result of these limitations, several studies have attempted to bolster fibrin-based approaches and largely focus on loading of growth factors into the fibrin glues to promote enhanced healing of the ACL. In one example, connective tissue growth factor (CTGF), known for stimulating ECM and vascular endothelial growth factor (VEGF) production, was introduced via dissolution in fibrin glue into the bone tunnels used for suture repair of a partially torn ACL in the rabbit model (P. Sun, Chen, Liu, & Gao, 2018; X. Sun et al., 2017). After two weeks, CTGF treatment significantly increased mRNA levels of transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1), collagens I and II, Sox9, and TIMP-1 (Tissue Inhibitor of Metalloproteinase-1) within ACL tissue, while showing a decrease in MMP-13 mRNA and protein expression when compared to fibrin-only treatment. At two and six weeks, CTGF treatment also demonstrated a significantly higher maximum load and stiffness, as well as an improved collagen alignment. Separate work has tested the effects of loading fibrin glue with platelet-derived growth factor (PDGF) or TGF- $\beta$ 1 in a model of partial ACL injury in rabbits without suture repair (Fig 5A-E) (Kondo et al., 2005). After 24 weeks, histological analysis indicated that groups with no treatment or treatment with fibrin sealant-only developed thin synovium-like tissue, whereas fibrin sealant + growth factor treatment groups developed thicker synovium-like tissue. While each treatment group was significantly different from the uninjured control in terms of anterior knee translation or maximum load and stiffness of the ACL, the fibrin sealant + TGF- $\beta$ 1 group appeared to have a positive impact on healing compared with the other treatment groups in terms of biomechanical properties (Fig 5F).

## **PRP/PRF**

While fibrin glues are comprised of only the protein component of clots (fibrin), PRP and PRF products also incorporate platelets to bolster pro-healing properties for therapeutic effect



(Beyzadeoglu et al., 2020; Silva & Sampaio, 2009). PRP and PRF originate from both autologous and donor blood sources and their manufacturing processes require the concentration of platelets in whole blood samples via erythrocyte removal (Mohan et al., 2019). In one method of PRP manufacturing, the anticoagulant sodium citrate is added to whole blood and the solution is centrifuged at slow speeds to separate erythrocytes from plasma, followed by a faster centrifugation to separate the previous supernatant into layers of platelet-poor plasma (PPP) at the top and PRP at the bottom. To manufacture PRF, whole blood is drawn and quickly centrifuged without anticoagulants at high speed for separation into PPP on top, a PRF clot in the center, and erythrocytes at the bottom (Mohan et al., 2019). Like fibrin glues, PRP and PRF form hydrogels upon initiation of clotting. Due to the use of anticoagulants, PRP relies upon the addition of exogenous thrombin, calcium chloride, or other agents for clotting activation during application, while for PRF, the coagulation cascade and platelet activation begin during preparation steps prior to application (Madurantakam, Yoganarasimha, & Hasan, 2015). *In vitro* tests have demonstrated that PRP gels generally exhibit a burst-release profile for growth factors and other molecules and degrade within three days, while PRF meshes exhibit sustained release mechanics and maintain structural integrity past seven days (Dohan Ehrenfest et al., 2014; Kobayashi et al., 2016). Additionally, PRP and PRF products exist in many forms with different production protocols, complicating comparisons of efficacy across studies (Dohan Ehrenfest et al., 2014).

Several preclinical and clinical studies have assessed whether PRP enhances ACL healing. Interestingly, in one such study, PRP alone did not enhance ACL healing in a porcine model of ACL injury and suture repair (Martha M. Murray et al., 2009). In humans, Koch et al. explored the effects of using autologous conditioned plasma (ACP), a form of PRP, with concurrent trephination of the femoral ACL stump and suture repair of the ACL (Koch, Di Matteo, et al.,

2018; Koch, Mayr, et al., 2018). At the two-year follow-up for a study of a group of patients who had experienced partial ACL ruptures, three of the 24 patients encountered ACL repair failure and 71.4% of the remaining 21 patients had an average return to sport of 4.8 months with good activity scores and a significant reduction of femorotibial translation (2.5mm preop to 1.5mm postop). However, this retrospective clinical study did not include a control group (Koch, Di Matteo, et al., 2018).

Approaches to combine platelet products with scaffolding materials for ACL repair have also been described. Murray and colleagues have led a number of preclinical studies (Joshi, Mastrangelo, Magarian, Fleming, & Murray, 2009; Mastrangelo, Magarian, Palmer, Vavken, & Murray, 2009; Martha M. Murray & Fleming, 2013; Martha M. Murray et al., 2007, 2006) combining PRP with a collagen-based scaffold for ACL repair. These collagen scaffolds are typically fabricated from a natural collagen-rich extracellular matrix that is digested and lyophilized. The technique is performed by drilling tunnels into the femur and tibia and threading absorbable sutures into the tibial end of the torn ACL and up into the femur tunnel. Non-absorbable sutures are then threaded through the scaffold and into the tibia and femur tunnels to provide enhanced knee stabilization during healing. The scaffold is soaked in PRP prior to placement at the site of ACL injury. Interestingly, although collagen (Fleming, Magarian, Harrison, Paller, & Murray, 2010) or PRP (Martha M. Murray et al., 2009) alone do not improve outcomes in the porcine model after ACL repair, their combination does. For example, the use of collagen-platelet composites with suture repair for treating complete ACL tears in pigs enhanced the biomechanical and histological healing of the ACL (Joshi et al., 2009; Martha M. Murray et al., 2007).

More recent work by the same group has evaluated whether whole blood (and the natural platelet concentration within) could provide similar results to prior studies using PRP. Preclinical

research demonstrated that collagen-based scaffolds had greater synovial fluid degradation resistance than fibrin-based scaffolds, and that physiologic platelet concentrations may demonstrate superior functionality in this system compared to PRP (Fleming et al., 2010; Martha M. Murray et al., 2016; Palmer et al., 2011; Perrone et al., 2017). In a long-term preclinical (Martha M. Murray & Fleming, 2013) study, bioenhanced ACL repair using an ECM scaffold and autologous blood was compared to traditional reconstruction using soft tissue grafts. By 12 months, the ACL treated with bioenhanced repair had similar stiffness, yield, and maximum load to those treated with ACL reconstruction, but with fewer signs of osteoarthritis (Fig. 6).

A similar approach, in which blood is injected directly into the scaffold post-placement, has now proceeded into clinical trials under the name bridge-enhanced ACL repair (BEAR) (Fig. 6) (Martha M. Murray et al., 2020, 2016, 2019). At two-year follow-up, compared with standard ACL reconstruction, BEAR treatment demonstrated no significant differences in IKDC Subjective Scores and AP knee laxity, with significantly improved hamstring strength. Results of an additional study indicate that neither physiologic platelet concentration variability between patients nor secretion of IgG with epitopes specific to the bovine collagen scaffold have any statistically significant impact upon treatment outcomes after six months, though white blood cell concentration may have an effect for certain groups (Freiberger et al., 2020). However, the BEAR technique was found to have a failure rate twice that of ACL reconstruction, which will require monitoring at longer-term follow-up (Martha M. Murray et al., 2020). The impact of BEAR treatment on osteoarthritis incidence in humans has yet to be investigated, as it often takes several years for this pathology to manifest.

PRP has also been used as a vessel for therapeutic delivery of bioactive agents or cells. For example, Zheng *et al.* examined the effects of PRP and a *Sanguisorba officinalis* polysaccharide

(SOWPa), a Chinese herb known to enhance collagen deposition, on ACL fibroblasts (Zheng, Huang, He, Tan, & Lin, 2020). Another common PRP administration approach includes the addition of adipose-derived progenitor cells (ADPCs) or mesenchymal stem cells (MSCs) (often in the form of bone marrow aspirate concentrate (BMAC)). In the dog model, partial ACL tears were treated using intra-articular injection of BMAC-PRP or ADPC-PRP. Data for each group was not generally separated in this retrospective study, but joint effusion levels and the total pressure index (TPI%) significantly improved after 90 days compared with baseline, and no significant difference was found after 90 days between treated and contralateral legs. There were some cases of degenerative joint disease as well as seven reported failures (Canapp, Leasure, Cox, Ibrahim, & Carr, 2016).

In clinical studies, microfracture for bone marrow-derived multipotent MSC release using the healing response technique and activated PRP glue/BMAC injection for growth factor addition were performed at the site of primary arthroscopic suture repair of partial ACL tears in athletes. At five year follow-up (Gobbi, Karnatzikos, Sankineani, & Petrera, 2013), four of the 50 patients experienced ACL re-rupture. Of the remaining patients, 78% presented with a normal IKDC objective score, and the difference in anterior knee translation changed from 4.1 mm to 1.4 mm at pre-op and five-years post-op, respectively. Average return to sport period was 5.9 months. At 10 year follow-up (Gobbi & Whyte, 2018), 12 of 44 returning patients had experienced ACL re-rupture. Despite these ruptures, the average anterior tibial translation of the cohort was reduced from 1.4 mm at short-term follow-up to 0.9 mm at long-term follow-up. The Kaplan-Meier repair survival plot indicated a repair survival rate of 72.7% at 14 years. In a separate clinical study, complete ACL tears of 10 patients were treated via intraligamentous injection of autologous bone marrow concentrate (BMC) mixed with PRP and platelet lysate to introduce MSCs to the injury

site. Seven of 10 patients showed improvement via MRI signal intensity at an average of 3.7 months post-treatment. Additionally, all seven patients that completed pre- and post-treatment functional scales reported improvement (note: not all patients completed all assessments in this study) (Centeno, Pitts, Al-Sayegh, & Freeman, 2015). While most recent clinical reports support the application of stem cells to PRP to promote intra-articular ligament repair, a study using the previously mentioned BEAR approach in the porcine model suggested that the addition of stem cells do not significantly benefit healing (Proffen et al., 2015).

While PRF has not been used for ACL healing in the context of repair, recent PRF studies focus on the application of PRF to bone tunnel healing and graft integration in ACL reconstruction. Although PRP and PRF have been shown to promote fibroblast proliferation and collagen production *in vitro* and improve ACL graft ligamentization and mechanics *in vivo*, the myriad production platelet concentrations and techniques used, the various application methods and sites (intra-articular, intra-ACL or intra-graft injection; bone tunnel or scaffold application), and mixed experimental results have led to much controversy, particularly in clinical outcomes (Andriolo et al., 2015; Beyzadeoglu et al., 2020). A recent editorial commentary explains this controversy succinctly and debates the value of pursuing platelet-rich products as surgical adjuvants for ACL reconstruction (Figueroa, 2020). The conflicting results of these recent studies and lack of PRF ACL repair research indicate that further investigation is required in this field.

## **Conclusions**

The ACL is critical for knee stability, and its poor vascularity and the surrounding thrombolytic synovial fluid environment prevents its healing following injury. Fibrin-based therapy options using fibrin glue, PRP, and other blood products are being increasingly explored

to supplement current ACL repair techniques. Indeed, the BEAR method shows promise in restoring joint stability while limiting osteoarthritis in animal models relative to ACL reconstruction. In the years to come, long-term results of the BEAR method and other fibrin-based treatments are needed to ensure limited ACL failure rates and cartilage damage.

Moreover, while current fibrin-based approaches show promise, surgical intervention is still required for administration. Non-surgical treatment such as via injectables may be a superior clinical option in cases such as partial tears if healing could be stimulated, thereby alleviating the need for costly surgery and post-operative complications while maintaining the original ACL tissue. Intra-articular knee injections are routinely performed in the context of OA pain management (Bellamy et al., 2006; Kon et al., 2011), lowering the barrier to clinical use.

Fibrin-based materials are poised to fill this gap, because they can be easily injected. However, many fibrin-based materials, including PRP, suffer from rapid degradation due to the harsh synovial environment, which may explain their lack of consistent data in the literature and subsequent lack of widespread clinical use. The recent development of materials that augment fibrin stability and possess the ability to simulate the natural clotting process, such as synthetic platelets, could be advantageous over other approaches such as biological clotting (e.g., PRP) as they address both clot formation and stability (Brown et al., 2014; Nandi et al., 2019). To date, such approaches have not been explored in the context of ACL repair. Fibrin scaffolds mixed with additional components also have potential to improve the effectiveness of ACL repair. Indeed, it is suggested that an advantage of the BEAR approach is that the collagen scaffold interacts with the blood to resist degradation (Palmer et al., 2011).

The use of biologics (including fibrin-based biomaterials) is a highly-disputed subject in the context of ACL injury treatment and its benefits and detriments are largely unknown. This

review revealed the need for well-controlled and mechanistic in vitro, preclinical, and clinical studies for proper assessment of the role of fibrin-based approaches in ACL healing. Additionally, the long-term impact of these therapies on knee stability and osteoarthritis are only beginning to be elucidated. Novel synthetic fibrin-based materials could be one of the next-generation treatments for improving ACL repair therapeutic potential.

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**Conflict of Interest:** Dr. Brown is a Founder and CEO for Selsym Biotech, Inc. which develops injectable hemostatic materials. Dr. Fisher has nothing to disclose. Mr. Scull has nothing to disclose.

## Tables

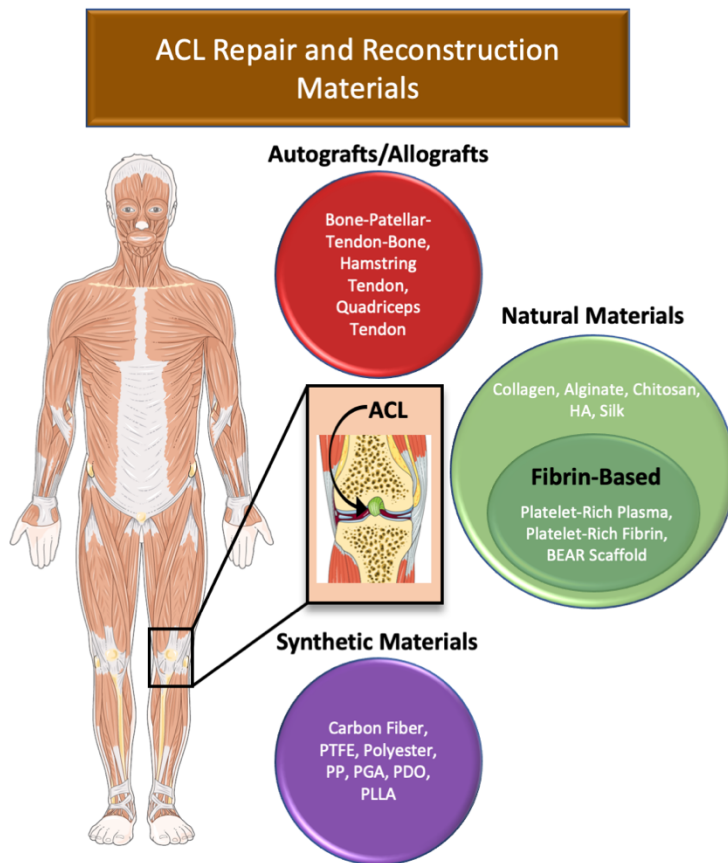
Abbreviation	Term
ACL	Anterior Cruciate Ligament
OA	Osteoarthritis
HA	Hyaluronic Acid
PTFE	Polytetrafluorethylene
PGA	Polyglycolic acid
PDO	Polydioxanone
PP	Polypropylene
PLLA	Poly L-lactic acid

ECM	Extracellular Matrix
MMP-1	Matrix Metalloproteinase 1
FDP	Fibrin Degradation Product
PRP	Platelet-Rich Plasma
PRF	Platelet-Rich Fibrin
CTGF	Connective Tissue Growth Factor
VEGF	Vascular Endothelial Growth Factor
TGF- $\beta$ 1	Transforming Growth Factor-Beta 1
TIMP-1	Tissue Inhibitor of Metalloproteinase-1
PDGF	Platelet-Derived Growth Factor
PPP	Platelet-Poor Plasma
ACP	Autologous Conditioned Plasma
BEAR	Bridge-Enhanced ACL Repair
ADPC	Adipose-Derived Progenitor Cell
MSC	Mesenchymal Stem Cell
BMAC	Bone Marrow Aspirate Concentrate

**Table 1. Abbreviations Used**

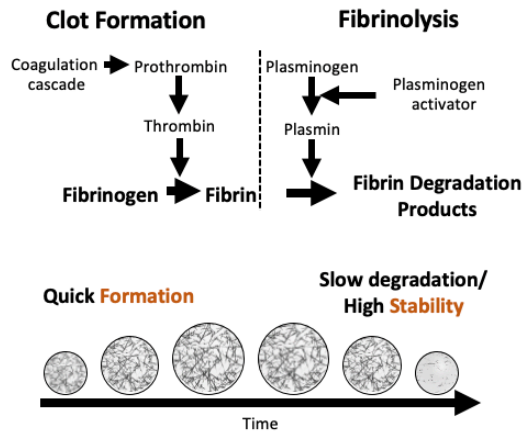


## Figures



**Figure 1. Anterior Cruciate Ligament location and repair/reconstruction materials.** Some models reproduced from SMART Servier Medical ART under Creative Commons Attribution 3.0.

## Normal Clotting in Plasma (Extra-Articular)



## Clotting in Synovial Fluid (Intra-Articular)

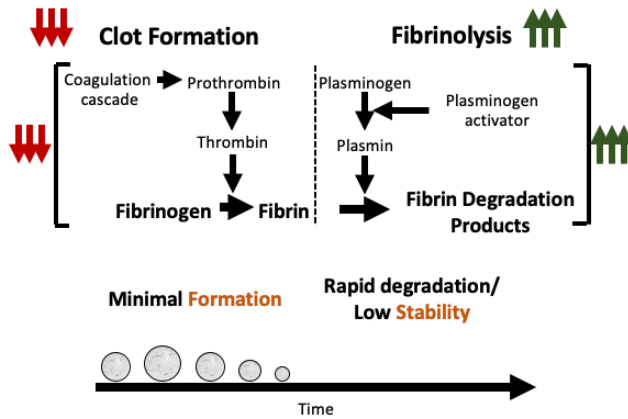
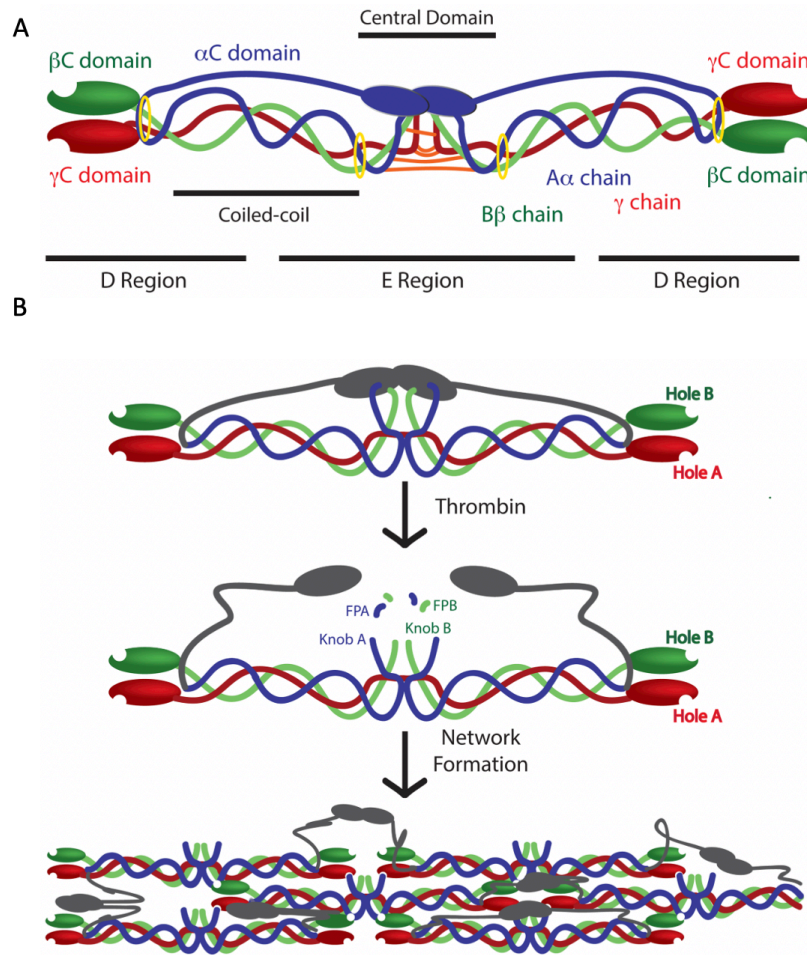
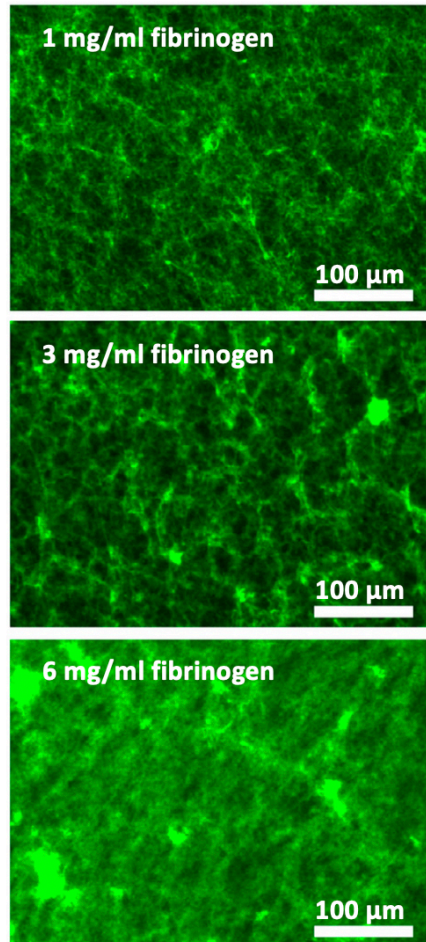


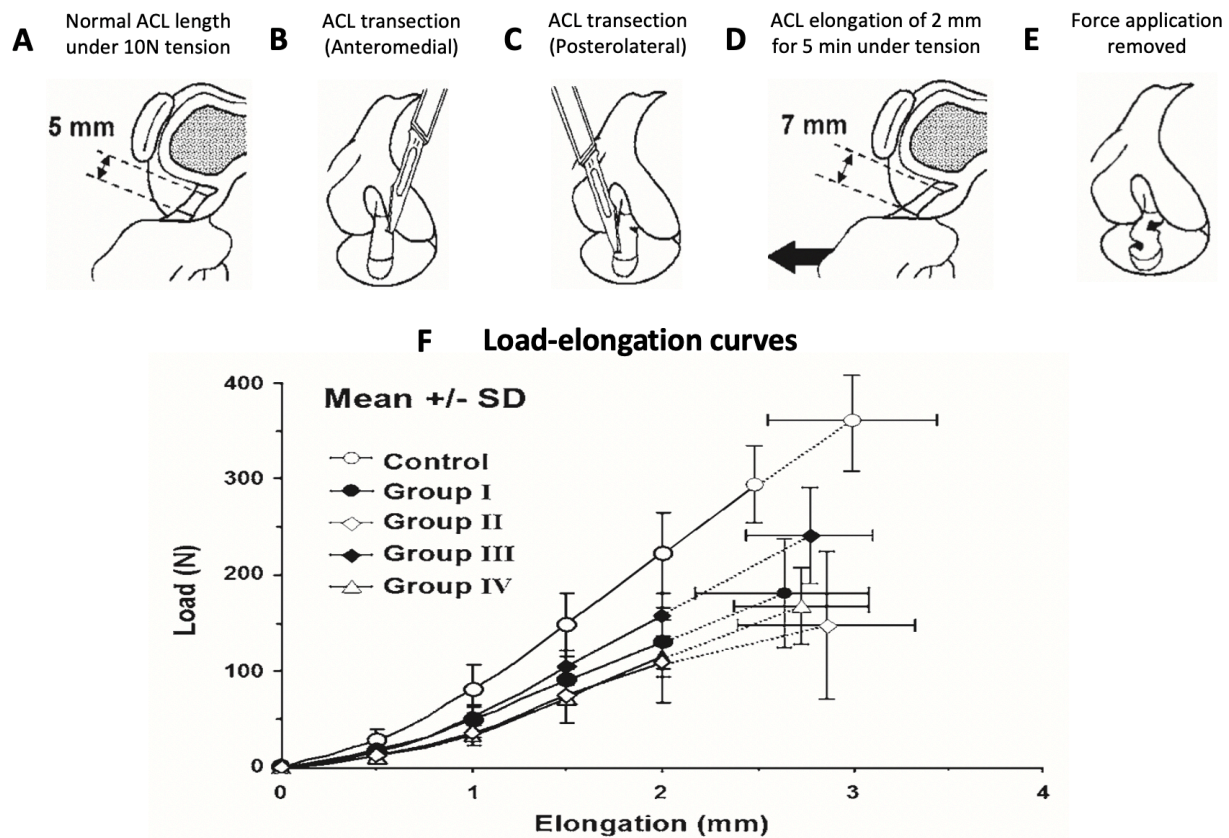
Figure 2. Normal clot formation and stability, and complications of the synovial environment.



**Figure 3. Overview of the fibrinogen molecule, its cleavage by thrombin, and subsequent polymerization into a fibrin network. (A) Fibrinogen Structure.** A $\alpha$  chains are shown in blue, B $\beta$  chains are shown in green, and  $\gamma$  chains are shown in red. Interchain disulfide bridges connecting the six polypeptide chains in the central domain are shown in orange and disulfide rings stabilizing the coiled coil regions are shown in yellow. **(B) Fibrin Polymerization.**  $\alpha$  chains are shown in blue,  $\beta$  chains are shown in green, and  $\gamma$  chains are shown in red.  $\alpha$ C domains are shown in gray. Reproduced from reference Brown et al. with permission from Elsevier, copyright (2014).



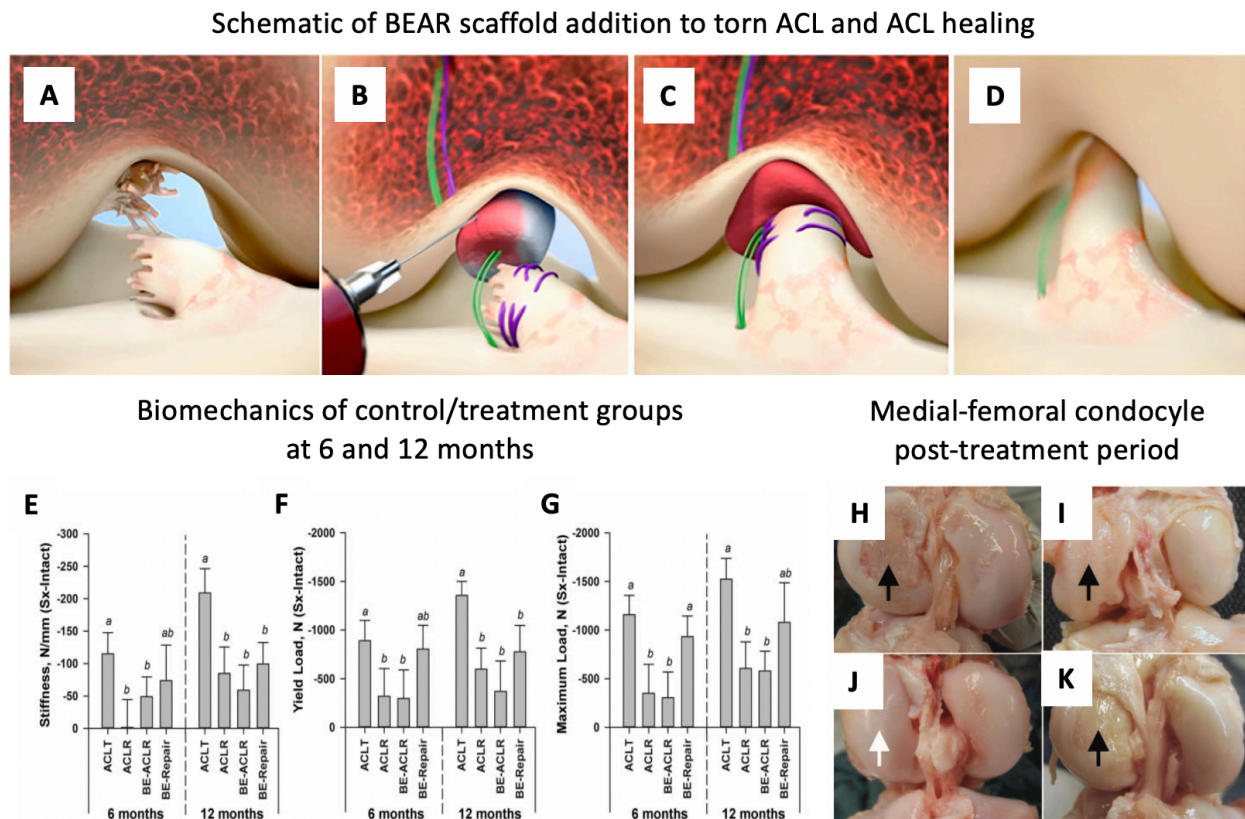
**Figure 4. Representative areas of FITC-labeled clots formed with three different concentrations of fibrinogen, illustrating decreasing porosity from low (1 mg/ml and 3 mg/ml) to high (6 mg/ml) fibrinogen concentrations.** (100x magnification, size bar represents 100 μm). Reproduced from reference Vavken et al. with permission from Elsevier, B.V., copyright (2009).



**Figure 5. Evaluation of fibrin-glue + growth factor treatments in a rabbit partial-ACL transection model.** (A-E) Operative treatment to create the elongation-type ACL injury with laceration. (A) The distance between the two lines was measured at 90° of knee flexion under an anterior drawer force of 10 N. The distance was approximately 5 mm. (B) and (C) The anteromedial and posterolateral halves of the right ACL were transected with a scalpel at the proximal and distal one-third levels, respectively. (D) A surgeon manually applied an anterior drawer force, monitoring the length between two marker lines, so that the ACL was elongated 2 mm for 5 minutes. (E) The ACL became slack after the anterior drawer force was removed. (F) Load-elongation curves for the femur-ACL-tibia complexes. Group 1- no treatment; Group 2- fibrin sealant; Group 3- TGF- $\beta$ 1 + fibrin sealant; Group 4- PDGF-BB + fibrin sealant. Each error



bar represents the standard deviation. Reproduced from reference Kondo et al. with permission from SAGE Publications, copyright (2005).



**Figure 6. Surgical procedure for the BEAR ACL repair technique and evaluation of outcomes 6-12 months after bioenhanced ACL repair in a porcine ACL transection model.**

(A-D) Stepwise demonstration of the bridge-enhanced ACL repair (BEAR) technique using the BEAR scaffold. (A) In this technique, the torn ACL tissue is preserved. (B) A whipstitch using No. 2 Vicryl (purple) is placed into the tibial stump of the ACL. Small tunnels (4 mm) are drilled in the femur and tibia, and an Endobutton with two No. 2 Ethibond sutures (green) and the No. 2 Vicryl ACL sutures attached to it is passed through the femoral tunnel and engaged on the proximal femoral cortex. The Ethibond sutures are threaded through the BEAR scaffold, tibial tunnel, and secured in place with an extracortical button. The BEAR scaffold is then saturated with 10 ml of

the patient's blood, and (C) the tibial stump pulled up into the saturated scaffold. (D) The ends of the torn ACL then grow into the BEAR scaffold and the ligament reunites. Reproduced from reference Perrone et al. with permission from Wiley Periodicals, Inc., copyright (2017). (E-G) The mean differences between limbs (surgical [Sx]-intact) for (E) linear stiffness, (F) yield load, and (G) maximum load for the 4 experimental groups at 6 and 12 months. The mean data are plotted with the 95% confidence intervals. A value of zero indicates that the yield or maximum failure loads are equal between legs. Means that do not differ between groups after Holm adjustment within each time point have the same lowercase letter (*a* or *b*). ACLT, ACL transection; ACLR, ACL reconstruction; BE-ACLR, bioenhanced ACL reconstruction; BE-repair, bioenhanced ACL repair. Reproduced from reference Murray et al. with permission from SAGE Publications, copyright (2013). (H-K) The distal femoral cartilage 1 year after (H) an untreated ACL rupture, (I) conventional ACL reconstruction, (J) bioenhanced ACL repair, and (K) bioenhanced ACL reconstruction. Note the damage to the medial femoral condyle in the untreated knee, ACL-reconstructed knee, and the bioenhanced ACL-reconstructed knee (black arrows) and the lack of damage to the medial femoral condyle in the bioenhanced ACL-repair knee (white arrow).

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