The past, present and future of ligament regenerative engineering

Regenerative engineering has been defined as the convergence of Advanced Materials Sciences, Stem Cell Sciences, Physics, Developmental Biology and Clinical Translation for the regeneration of complex tissues and organ systems. Anterior cruciate ligament (ACL) reconstruction necessitates the regeneration of bone, ligament and their interface to achieve superior clinical results. In the past, the ACL has been repaired with the use of autologous and allogeneic grafts, which have their respective drawbacks. Currently, investigations on the use of biodegradable matrices to achieve knee stability and permit tissue regeneration are making promising advancements. In the future, utilizing regenerative biology cues to induce an endogenous regenerative response may aid the enhancement of clinical ACL reconstruction outcomes.

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Anterior cruciate ligament injuries, repair limitations & surgical intervention

Annually, over 400,000 anterior cruciate ligament (ACL) reconstructions are performed worldwide [1]. In the USA alone, as many as 250,000 ACL injuries occur each year that necessitate surgical intervention, at an annual healthcare cost upward of US\$18 billion [2]. Generally, injury to the ACL occurs during sporting activities with pivoting and quick stop and go actions. During these activities a 'point of no return' may occur when high tensile and torsional forces are placed on the ACL. Unfortunately, the ACL does not have the capacity for intrinsic healing. The synovium environment that surrounds the ACL inhibits the formation of a blood clot and leaves patients with an unstable knee [3]. In the case of extra-articular ligaments, such as the medial cruciate ligament, an endogenous healing response occurs through the formation of a blood clot, which serves as a scaffold to allow healing of the ruptured ligament without the need for surgery. The lack of a provisional scaffold for ACL heal-

ing means surgical intervention is needed to stabilize the knee and regain its kinematic function.

Present methods to reconstruct a torn ACL utilize autografts and allografts. Autografts and allografts that are used clinically are derived from hamstring tendon, semitendinosus quadriceps and bone-patellar tendon-bone grafts (BPTB) [4–6]. Comparisons between the graft choices have shown that BPTB grafts have superior bone integration than hamstring and semitendinosus quadriceps grafts [7]. Also, it should be noted that the BPTB graft is in fact a ligament, since the common nomenclature of a ligament is the attachment of bone to bone. In contrast, tendon is defined by its attachment of bone to muscle. This discrepancy may be a logical reason for the improved graft incorporation of the BPTB grafts, as it is a natural ligament and incorporates the natural interface needed for an ACL. Given the clinical efficacy of BPTB autografts, it is considered to be the gold standard treatment for ACL reconstruction. Yet, drawbacks exist for BPTB grafts, namely donor-site morbidity. The patellar

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tendon is harvested from the middle third of the ligament that connects the patella and tibia, which allows for the continued function of the patella tendon. Nevertheless, patients that undergo BPTB harvest are known to suffer from acute knee pain when kneeling, which is a consideration when choosing this graft [4]. Furthermore, allografts are predominately used in secondary ACL reconstruction, in which the BPTB has been previously harvested, but the limitations in supply and risk of disease transmission are drawbacks [8].

Although the gold standard treatment of ACL reconstruction achieves knee stability, drawbacks exist: donor-site morbidity, patient to patient variability in graft strength and the necessity for approximately 8 months of rehabilitation prior to return to sport activity. The focus of ACL reconstruction is to stabilize the knee joint in the short and long term, and the choice of graft is determined by the ability of the graft to handle the physiological tensile loads placed on the ACL during normal activity. The shortcomings of current auto-

Figure 1. Anterior cruciate ligament reconstruction techniques. (A) Final fixation of bone–patellar tendon–bone graft with single-bundle anterior cruciate ligament (ACL) reconstruction technique. (Reproduced with permission from [9]). (B) Fixation of semitendinosus hamstring graft utilizing a double-bundle ACL reconstruction technique. (Reproduced with permission from [10]). (C) Bioenhanced ACL repair. (Ci) Femoral and tibial tunnels are drilled and an endobutton is fixed on the femoral cortex. Three sutures are utilized and result in six free strands. (Cii) A collagen scaffold is threaded onto the four red strands, while a Kessler suture is placed on the tibial ACL stump. The collagen scaffold is pushed into the notch of the femoral insertion, and saturated with platelet-rich plasma. (Ciii) The four sutures (red) are passed through the tibial tunnel and fixed with an endobutton. (Civ) Finally, the Kessler sutures are knotted to the collagen–platelet composite. Adapted with permission from [11]

grafts and allografts have spurred investigation into the possibilities of synthetic bioengineered ACL matrices to improve the immediate stability of the knee joint and accelerate the rehabilitation of patients.

Framework to define regeneration of the ACL

Anatomy

In order to design matrices that mimic the native ACL, an understanding of the complex hierarchical structure of the soft tissue must be met. The human ACL is approximately 27–32 mm in length and has a cross-sectional area of 44.4–57.5 mm² [12,13]. Macroscopically, the gross structure of the ACL appears as a band-like structure with two bundles, the anteromedial (AM) and the posterolateral (PL) bundles, which connect the femur and the tibia [14]. The AM and PL bundles are defined by the location of their insertion into the tibial plateau. The ACL travels anteriorly, medially and distally from the femur to the tibia, and is characterized by a 180 degree twist between its bony attachment ends [14]. Functionally, the AM and PL bundles have contrasting orientations that are dependent on the movement of the knee joint. Thus, these two bundles are fundamental for the biomechanics of dynamic knee movement. Engineering synthetic ACL matrices should take into account the role of the AM and PL bundles during the dynamic movement of the knee joint (torsion and pivoting).

On a cellular and extracellular matrix level, ligaments are dense and complex tissues composed of collagens (type I, III and V), elastin, proteoglycans, water and cells [13,14]. Collagen molecules, fibrils, fibril bundles and fascicles are arranged parallel to the longitudinal axis of the ligament and compose its hierarchical structure [15]. The ACL can be divided into three sections: proximal, mid-substance and distal. Cellular and extracellular matrix components differ in each of these sections. The proximal section has the greatest cellularity, and is comprised primarily by fibroblasts, type II collagen and glycoproteins [14,16–18]. In the middle section lie spindle-shaped fibroblasts, a high density of collagen fibers and a special zone of cartilage and fibrocartilage located at the ligament–bone interface [14]. The longitudinal orientation of the fusiform and spindle-shaped fibroblasts in the middle zone is of critical importance to ACL function. The cellular organization in the mid-substance allows for the collagen fibrils to be deposited in a crimp-like pattern, which contributes to the nonlinear stress–strain response of the ACL. Finally, the distal section of the ligament is rich in chondroblasts, has a lower density of collagen bundles and is described to be more ‘solid’ than the aforementioned sections. In the anterior por-

tion of the ACL, a layer of dense fibrous tissue engulfs the ligament. The tissue that engulfs the ligament is compressed by the anterior rim of the femoral intercondylar fossa, and may have a functional role in protecting the ligament. The diversity of the ACL sections provides insight into the difficulty of recapitulating its protective features with reconstruction: proximal site allows for stress distribution from the mid-substance to bone; the crimp pattern inherent to the mid-substance allows for a nonlinear stress–strain response; the distal site may protect the bulk of the ligament from impingement with the femoral intercondylar fossa.

Biomechanics

The mechanical properties of the ACL are evaluated as a combination of the ligament, the insertion site and its fixation. In cadaveric studies, Woo *et al.* evaluated the structural properties of the femur–ACL–tibia complex and reported a mean ultimate load of 2160 N and a mean stiffness of 242 N/mm for specimens in the age of 22–35 years old [19]. Additionally, the mechanical properties of BPTB and quadrupled hamstring grafts were evaluated. The quadrupled hamstring graft has superior biomechanical properties, a mean ultimate load and stiffness of 2422 N and 238 N/mm, respectively, and the BPTB has a mean ultimate load of 1784 N and a mean stiffness of 210 N/mm. The biomechanical properties of the ACL are generally described by its tensile strength, and the native human ACL exhibits a tensile strength of 1800–2196 N [6]. Therefore, synthetic ACL matrices should aim to recapitulate the native mechanical properties of the ACL at the time of implantation and throughout its lifetime.

The structure and components of the ACL work in unison to provide the biomechanical nonlinear stress–strain response are seen in the intra-articular region of the ligament. The organizations of the collagen fibrils are parallel and dense, and the combination of the helical, planar and parallel organization gives rise to the biomechanical response of the ligament [16,17,20]. At the level of fascicles, a planar wave-like form is present, which is analogous to a crimp pattern and endows the matrix with a defined sinusoidal pattern. Upon elongation of the ACL under tensile stretch, the crimp pattern is first straightened out and then followed by fibril elongation. As increasing numbers of fibrils experience tensile strain, the ACL gradually stiffens in a linear fashion. Further loading of the ACL, after fibril elongation, causes the ligament to plastically deform to its yield point. In addition, collagen fibrils themselves have a hierarchically organized substructure, which is composed of collagen molecules that are in the form of a triple-helix [21,22]. This hierarchy of the ligament components well describes the stress–strain relationship of

the intra-articular ligament. The importance of recapitulating the nonlinear stress–strain behavior of the ACL in newly designed synthetic ligaments is to provide a gradual transfer of load from the intra-articular joint to the cells to eliminate stress-shielding [23].

The ligament–bone interface

The correct healing of this interface is critical to the outcome of ACL surgical reconstruction. As a transitional zone, the ligament–bone interface not only serves to gradually distribute stresses, but to also control the fixation of the ligament. Improper healing of this site can lead to a loosely fixed ACL that continuously causes the ligament and bone tunnel to rub against each other and undergo greater stress concentrations [16]. This can lead to an overall change in the biomechanics of the ACL that leads to degradation of the bone tunnel and ligament proper.

The importance of the interface is due to the discrepancy between the elastic modulus of bone and ligament; the elastic modulus of bone is approximately ten-times greater than that of ligament [24]. Thus, the interface gradually changes from ligament to fibrocartilage and then to rigid bone to allow for the distribution of stresses due to mechanical loading of the ACL. Although several attempts have been made to study the biomechanical aspects of the interface in relation to its junctional properties, there has been limited success due to the experimental difficulties in recording strain levels within the small volume of the region and the inability to clearly define the transitional zones within the interface [25,26]. It has been hypothesized that the loading of the interface is nonuniform. This theory of stress distribution is guided by the knowledge that the pathology of the interface occurs in the regions that experience the lowest levels of stress [24,26]. Furthermore, the clinically recognizable abnormalities of the interface most frequently occur in the deep zone as opposed to the superficial region, which corresponds to the greater presence of fibrocartilage in the deep zone. The localization of fibrocartilage in the interface is due to its functional role, to act as a shock absorber when compressive loads are applied, and that the deep zone is compressed by the superficial zone. Mineralized fibrocartilage represents the final transition to rigid bone. At this point the surface area of the interface is increased between the ligament and bone to promote a firm anchorage and resist shear stresses [26]. Overall, the characteristic of the interface is composed of a cartilage-like substance.

Past synthetic ligament matrices

Since the 1970s, the use of synthetic nondegradable ACL matrices for ACL reconstruction has been uti-

lized clinically along with surgical techniques for optimal stability of the knee joint [27]. Synthetic nondegradable ACL replacements are either augmentation devices or permeant replacements. An augmentation device is defined as a prosthetic that is utilized in conjunction with autologous and/or allogenic grafts. One of the first US FDA approved augmentation devices is the 3M Kennedy ligament augmentation device (3M, MN, USA), which was approved in 1987, and the utilization of this graft was accompanied by the Marshall–MacIntosh technique [28]. Since then, several permanent synthetic nondegradable devices have been used clinically: carbon fiber braided matrices, the Gore-Tex Device (W.L. Gore, AR, USA) and the Dacron Device (Stryker, MI, USA) [1]. The aim of these permanent and augmentation synthetic ligament prostheses was to counteract the drawback of autografts, donor-site morbidity, and to allow for a more stable knee joint in the early postoperative phase. The goal of these procedures is to accelerate patient rehabilitation to allow patients to return to prior activity levels quicker and safely.

The use of permanent synthetic nondegradable matrices has not generated suitable efficacy to recommend clinical use. Types of failure of nondegradable matrices include creep, knee effusion and synovitis, presence of wear particles in the joint and lymph nodes, and premature osteoarthritis [29]. In the early 1980s, the Leeds-Keio device (Xiros [formerly Neoligaments], Leeds, England) produced considerable interest because of its ability to promote tissue ingrowth, thus alleviating stress-shielding effects as the graft matured [27]. Yet, several other permanent devices did not permit tissue ingrowth and suffered from cyclic fatigue, which lead to laxity of the knee.

Present approaches & materials for ligament regenerative engineering

The American Society for Testing Materials has categorized synthetic ACL matrices as Tissue Engineered Medical Products (TEMPs) [30]. The American Society for Testing Materials standards for TEMPs state that their use is contingent on the intent to improve surgical outcome by assuming mechanical load at the insertion site, improving biological healing or a combination of the two elements. Furthermore, TEMPs should improve clinical outcome. In order to evaluate the efficacy of new synthetic matrices, preclinical animal models are employed. Currently, the ovine model is often used to evaluate the efficacy of ACL reconstruction procedures, due to the strong similarities between the stifle and human knee joint [31].

ACL reconstruction surgical techniques include single- and double-bundle repair, as well as primary repair

with suturing (Figure 1). The single-bundle technique utilizes a BPTB graft, which is fixed into the femoral and tibial tunnels with either interface screws, endobuttons or a combination of the two (Figure 1A). The shortcoming of this technique is that it does not recapitulate the native anatomy of the ACL, which is comprised of an AM and PL bundle. Therefore, researchers have looked into the use of a double-bundle reconstruction technique to more closely mimic the native ACL (Figure 1B).

In a recent randomized controlled clinical trial, the results of single-bundle versus double-bundle ACL reconstruction were evaluated [5]. Seventy-eight patients were enrolled in the study, and 53 were evaluated for 3 years. Through the evaluation of traditional physical examinations, double-bundle reconstruction was shown to perform better when assessed with the Lachman and pivot-shift test, as well as KT-1000 arthrometer measurements. Overall, the study demonstrated that double-bundle reconstruction was significantly better in terms of anterior and rotational stability.

Primary ACL repair sutures the ruptured ends of the ACL together to allow the tissue to heal. However, studies have reported unacceptable rates of failure after primary surgical repair, which is attributed to the intra-articular environment and synovial fluid that surrounds the ACL [32]. Murray *et al.* elucidated the differences in intra-articular (i.e., ACL) versus extra-articular (i.e., MCL) healing in a canine central ACL wound model. In the extra-articular healing process, a fibrin-platelet clot forms within the wound site to connect the ruptured ends of the tissue, and serves as a provisional scaffold to allow for tissue ingrowth and remodeling. In contrast, an upregulated production of urokinase plasminogen activator by synovio-cytes occurs in intra-articular ligament injuries, which converts plasminogen into its active form plasmin. Plasmin degrades fibrin, and the clot that serves as a provisional scaffold is lost.

The loss of a provisional scaffold is the predominant reason for the inhibition of tissue regeneration within joints, such as the ACL or meniscus [32–34]. Additionally, the lack of a provisional scaffold in the intra-articular ligament wound is associated with decreased levels of inflammatory cytokines that are needed for the healing response (fibrinogen, PDGF, TGF- β and FGF). To combat these issues Murray and associates developed a new approach toward primary ACL repair, which utilizes a collagen-platelet rich scaffold to act as a provisional scaffold. *In vitro* studies demonstrated that cells from an embedded ACL tissue could migrate into a collagen hydrogel, and that the addition of platelet concentrate increased proliferation and

collagen production rates [35–37]. In a porcine model, Vavken *et al.* devised a surgical procedure in which the surgeon sutures a collagen-platelet rich plasma scaffold to the ruptured ACL (Figure 1C) [11]. In comparison to BPTB allografts, no difference in maximum load, maximum displacement or linear stiffness was seen when utilizing the bioenhanced ACL repair technique. The promising results in large animal models have led to the first clinical safety study in humans [38].

The clinical efficacy of the bioenhanced primary ACL repair has yet to be realized. Currently, in clinic settings, the use of single-bundle or double-bundle ACL reconstruction is commonly used to stabilize the knee post ACL rupture. Augmentation devices, such as the ligament advanced replacement system (LARS), are also used and have shown a high success rate. Present generation synthetic materials for ligament regenerative engineering include, poly(L-lactic acid) (PLLA) [12,29], poly(glycolic acid) (PGA) [39], poly(lactic-co-glycolic acid) (PLGA) [29], polycaprolactone [40] and silk [41,42]. These materials are biodegradable, and thus allow for the regeneration of the ACL over time while providing initial stability of the knee joint. By tuning the degradation rate of these materials, tissue ingrowth can be facilitated without compromising mechanical strength. Furthermore, ligament scaffolds may be chemically modified to incorporate adhesion moieties, such as fibronectin or Arg–Gly–Asp peptides to improve cell proliferation and extracellular matrix production [29].

Currently, the most successful augmentation device is the LARS. The device utilizes the ruptured ACL stump in conjunction with a polyethylene terephthalate (PET) matrix to stabilize the knee. A recent systematic review of synthetic devices found that the failure rate of the LARS device was 2.6% out of 736 patients that were included in the studies inclusion criteria [43]. Furthermore, the LARS device allowed patients to return to unrestricted activity after 2 months in one study, 3 months in three studies, 4 months in four studies and 6 months in two studies. The ability to shorten the rehabilitation time of patients can make a marked impact economically. As rehabilitation time is decreased, so are the healthcare costs that go toward physical therapy for patients. However, as Batty *et al.* stated, the results of the LARS should be considered with caution as the definition of ligament failure is perceived with different interpretations from study to study [43]. The LARS ligament is made from polyethylene terephthalate, which is a nondegradable material. Inherently, nondegradable materials do not allow for regeneration; the synthetic material is not an intrinsic property of the native ACL. To achieve regeneration, biodegradable materials as a synthetic ligament replacement have been utilized.

Silk is a natural biodegradable material that has been well studied for ACL matrices. Silk fibers have been widely used as a clinical suturing material, due to its favorable biocompatibility and mechanical strength [42]. In a study by Altman *et al.*, silk fibers were braided in a 6-cord arrangement, which provide mechanical properties similar to the human ACL. Additionally, the slow rate of degradation of silk is a desirable quality and theoretically allows for the gradual transfer of mechanical load from the graft to the neoligament [41]. In the past, the drawback of the use of silk is the immune-eliciting sericin proteins, and that the degumming processes significantly alter the biomechanical properties of silk scaffolds. However, Teuschl *et al.* recently demonstrated that the use of a borate buffer-based system can effectively remove sericin proteins while maintaining the mechanical properties and biocompatibility of braided silk scaffolds [44]. Furthermore, Teuschl *et al.* evaluated the suitability of silk synthetic ligaments that was braided into a wire rope-like structure with an outer sheathing element, for ACL reconstruction in a large animal study [41]. The study found that the silk matrix initiated ligament regeneration and that silk degradation occurred in parallel to the regeneration of tissue.

PLLA is an attractive synthetic polymer for ligament regenerative engineering. Composed of lactic acid monomers, the degradation of PLLA occurs through hydrolysis and has been reported to take approximately 2 years to degrade. A 2-year time point for degradation permits tissue infiltration while maintaining mechanical integrity. Lu *et al.* investigated the use of poly(glycolic acid), PLLA and PLGA scaffolds for ligament regenerative engineering [29]. The study indicated that PLLA-based scaffolds maintained long-term structural integrity and exhibited superior mechanical properties. Furthermore, the study concluded that PLLA was the most suitable material for ligament regenerative engineering due to its favorable effects on cellular proliferation when compared with PLGA scaffolds.

3D braiding technology allows for the development of a structure with continuous interconnected pores and an increased surface area, which permits greater cell attachment and enhanced regeneration through tissue ingrowth. Additionally, braiding allows for the development of mechanically robust structures that can recapitulate the biomechanical properties of the human ACL. Knitting techniques for ACL matrices have also been explored, as they can provide greater internal connective spacing for tissue ingrowth [45]. However, the mechanical properties of knitted matrices may not be suitable for clinical translation and have been used in conjunction with braided matrices to enhance strength [46]. Finally, 3D braiding technology

may also offer the ability to generate pore size gradients that can vary radially, which can have implications in eliciting greater cell infiltration [47].

Given the advantages of 3D braiding technology, Cooper *et al.* developed a PLLA 3D braided construct that was optimized for bone integration and ligamentization [15,23,48]. The 3D braided construct was composed of two bony attachment zones, which are braided with a higher braiding angle than the intra-articular zone of the scaffold (Figure 2A). By controlling the braiding angle, the porosity of the bioengineered matrix could be tuned for optimal bone and soft tissue ingrowth. The pore sizes of the bony ends were in the range of 150 μm , 200–250 μm in the intra-articular region, and the bioengineered matrix has an overall porosity of >50% [49]. Additionally, PLLA microfibers were utilized to mimic the diameter of collagen fascicles present in the native ACL. In a rabbit model, the efficacy of the braided construct was investigated at 4 and 12 weeks showing the ability of the construct to allow for bone tissue infiltration and ligamentization [23]. Additionally, ACL fibroblasts were seeded on the bioengineered scaffold, prior to ACL reconstruction, and were observed to enhance tissue growth. A follow-up study in an ovine large animal model demonstrated favorable results for ACL regeneration. As seen in Figure 2B, the bioengineered ligament demonstrated similar gross features at 3 months post ACL reconstruction in comparison to the native ACL. At the 6-month time point, tissue ingrowth was visualized around the PLLA fibers (Figure 2C). Finally, 1.5 years post ACL reconstruction, complete regeneration of the intra-articular zone of the ACL was seen as denoted by the absence of the PLLA fibers and the crimp like pattern of the newly regenerated ACL tissue (Figure 2D) [50].

Conclusion

Synthetic materials hold great promise to develop ACL matrices for ACL reconstruction. The use of braiding technology has demonstrated the ability to develop robust matrices with mechanical properties that allow for initial knee stability. Furthermore, the ability to control the degradation of synthetic materials allows for long-term stability of the knee while regeneration persists. By adequately tuning the degradation of synthetic matrices to match the rate of extracellular matrix deposition, an ACL may regenerate while still providing the mechanical strength necessary to withstand physiological loads.

Future perspective

Given the promise of 3D biodegradable braided scaffolds, the future of ligament regenerative engineering

Figure 2. Anterior cruciate ligament reconstruction utilizing the L-C ligament. (A) 3D poly(L-lactic acid) square braid with bony attachment zones and an IA zone. (Reproduced with permission from [48]). (B–D) Gross observation and histological comparison of native anterior cruciate ligament (left column) and tissue regeneration on the 3D poly(L-lactic acid)-bioengineered ligament (right column). (B) Gross observation of the regenerated ligament in autograft group (left image) and bioengineered ligament (right image) 3 months postimplantation (arrow points to anterior cruciate ligament). (C) Intra-articular region of the autograph (left image) and bioengineered ligament (right image) after 6 month postimplantation. The bioengineered ligament shows tissue infiltration and presence of some fibers. (D) IA region of the bioengineered ligament graft after approximately 1.5 years postimplantation showing completely regenerated ligament with organized collagen fibers. The right image is of higher magnification and shows the crimp pattern in the tissue and presence of fibroblasts. BA: Bony attachment; IA: Intra-articular.

entails defining a regenerative niche for ACL regeneration. There is a need to determine a regenerative competent microenvironment that can elicit tissue ingrowth and regeneration. As a part of the microenvironment, identifying regenerative competent cells is of importance. The endogenous cells of the ACL that may contribute to ACL regeneration include syno-

vial stem cells, bone cells and cells migrating from the posterior cruciate ligament. The addition of stem cells onto ligament constructs may also aid to modulate the inflammatory response in the early phases of healing. Currently the use of stem cells to enhance tissue growth has been widespread; however, the efficacy of stem cell treatment is unclear. Several studies

Executive summary

Anterior cruciate ligament injuries, repair limitations & surgical intervention

- Annually, over 400,000 anterior cruciate ligament (ACL) reconstructions are performed worldwide.
- The ACL cannot heal without surgical intervention due to the avascularity of the tissue and the surrounding synovial environment.
- The gold standard for clinical treatment of ruptured ACLs utilizes bone–patellar tendon–bone grafts, semitendinosus hamstring or semitendinosus tendon grafts.
- Drawbacks of current ACL reconstruction techniques are donor-site morbidity, interpatient variability in graft strength and the necessity for approximately 8 months of rehabilitation prior to return to sport activity.
- Synthetic bioengineered ACL matrices may improve the immediate stability of the knee joint and accelerate patient rehabilitation.

Framework to define regeneration of the ACL

- The human ACL is comprised of two bundles, the anterior medial and posterolateral bundle, which are fundamental for the biomechanics of dynamic knee movement.
- On a cellular and extracellular matrix level, ligaments are dense, complex tissues composed of collagens (type I, III and V), elastin, proteoglycans, water and cells.
- The cellular organization in the mid-substance allows for the collagen fibrils to be deposited in a crimp-like pattern, which contributes to the nonlinear stress–strain response of the ACL.
- The biomechanical properties of the ACL are generally described by its tensile strength. The native human ACL exhibits a tensile strength of 1800–2196 N.
- In designing new ACL matrices, recapitulating the nonlinear stress–strain behavior of the ACL is of importance due to its role in providing gradual transfer of load from the intra-articular joint to the cells to eliminate stress shielding.
- The interface between the ligament and bone plays a fundamental role in distributing stresses from mechanical loading.

Past synthetic ligament matrices

- Since the 1970s, the use of synthetic nondegradable ACL matrices for ACL reconstruction has been utilized clinically, including carbon fiber braided matrices, the Gore-Tex Device and the Dacron Device.
- The failure of nondegradable matrices is contributed to creep, knee effusion and synovitis, presence of wear particles in the joint and lymph nodes, and premature osteoarthritis.

Present approaches & materials for ligament regenerative engineering

- ACL reconstruction surgical techniques include single- and double-bundle repair, as well as primary repair with suturing.
- Present generation synthetic biodegradable materials for ligament regenerative engineering allow for the regeneration of the ACL over time while providing initial stability of the knee joint. These materials include poly(L-lactic acid), poly(glycolic acid), poly(lactic-co-glycolic acid), polycaprolactone and silk.
- By tuning the degradation rate of biodegradable materials, tissue ingrowth can be facilitated without compromising mechanical strength.
- 3D braiding technology allows for the development of a structure with continuous interconnected pores and an increased surface area, which permits greater cell attachment and enhanced regeneration through tissue ingrowth.

Future perspective

- Given the promise of 3D biodegradable braided scaffolds, the future of ligament regenerative engineering entails defining a regenerative niche for ACL.
- regeneration that can elicit tissue ingrowth and regeneration.
- Controlling the degradation of synthetic materials may allow for the gradual transfer of mechanical load to regenerated tissue while allowing for long-term stability.
- Sulfated GAGs may have the potential to stimulate an endogenous regenerative response, and may aid in enhancing and accelerating ACL regeneration.

have explored the use of growth factors for ACL repair (GDF 5/6 [51], VEGF [52,53], PDGF [54], among others). However, another avenue to consider is the use of growth factor sequestering moieties to modulate the endogenous growth factors in the environment.

Heparin and heparan sulfate are well known for their ability to sequester growth factors and have recently demonstrated the ability to induce a regenerative response in the axolotl [55,56]. The results of the study demonstrate the potential for sulfated glycosaminoglycans (GAGs) to stimulate an endogenous regenerative response. Native to the ACL, as determined in a swine, is that chondroitin sulfate and dermatan sulfate constitute the main GAG components of the tissue [57]. Furthermore, keeping in mind the role of GAGs in the viscoelastic response of the

ACL, it may be beneficial to explore the opportunity of GAGs to induce a regeneration response.

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