Fibroin silk and ligament reconstruction: State of art and future prospective

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Introduction

The Anterior Cruciate Ligament (ACL) has long been considered the primary passive restraint to anterior translation of the tibia with respect to the femur. Moreover, it contributes to knee rotational stability in both frontal and transverse planes due to its specific orientation [1]. The high incidence of ACL tears, coupled with the absence of adequate clinical options to restore full knee joint function, have been the main focus of research over the past decades. Indeed, ACL lesions are one of the most common knee injuries mainly sustained as a result of sports participation. These injuries often result in joint effusion, altered movement, muscle weakness, reduced functional performance, and may lead to the loss of an entire season or more of sports participation among young athletes [2]. In addition, ACL injury often results in clinical sequelae including meniscal tears, chondral lesions, and osteoarthritis. For these reasons, several treatments have been proposed to restore the gross stability of a symptomatic ACL-deficient knee. ACL repair by reapproximating the two ends of the ruptured ligament using suture was one of the earliest suggested treatment described by Robson in the early 1900s [3]. Unfortunately, the high rates (40% to 100%) of failure of ACL healing, have led to abandonment of suture repair and almost universal adoption of ACL reconstruction through graft [2]. In ACL reconstruction, the torn ACL tissue is removed and replaced with an allo- or autograft tendon taken either from the medial hamstrings or the middle third of the patellar tendon. Although ACL reconstruction has become the current gold standard for restoring the central pivot, significant problems persist regardless the graft type. In the short term, conventional ACL reconstruction fails to restore the normal joint kinematics, mainly due to non-anatomic ligament insertion and alignment, loss of native proprioception, graft- tissue degeneration and neuromuscular deficit [5]. Autografts have the benefits of earlier incorporation and no rejection or disease transmission but require longer operative times and a potential donor site morbidity. On the other hand, allografts have the advantages of decreased donor site morbidity, availability of multiple grafts and shorter operative times, but carry a small but significant risk of disease transmission. For these reasons, over the last decade, substantial effort has been made to propose the ideal graft characterized by rapid incorporation, low failure rates, high degree of safety, low donor site morbidity, wide availability, and low cost, but to date, a graft with all these characteristics does not yet exist [6]. Because of these ongoing issues with ACL reconstruction, there is a need to develop new approaches that may lead to better outcomes. The role of the scaffold needs to include two different interrelated scales: The macroscopic scale at which the scaffold should meet the anatomical dimensions of the replaced tissue, and should fulfil its physiological function.
during the rehabilitation period; the microscopic scale at which the scaffold should promote tissue formation by providing the cells with a suited micro-environment.

Finding the right biomaterial that may function as a potential scaffold is one of the key challenges. Indeed, it needs to be biocompatible, biodegradable to allow the tissue ingrowth and have mechanical properties as close as possible to the natural ACL to provide immediate mechanical stability after implantation [7]. Silk has been extensively used in clinical practice as a suture material and has recently gained renewed interest as a biomaterial for tissue engineering. Silk Fibroin (SF) is characterized by high biocompatibility, controllable biodegradability, low immunogenicity and limited pathogen transmission. In addition, it also owns excellent mechanical properties and structural integrity [8]. This review article is focused on recent research based on silk fibroin in the field of ligament regeneration and evaluates its prospects for further development in therapeutic related applications.

**Anatomy and biomechanics of anterior cruciate ligament**

The ACL has been the focus of many biomechanical/anatomical studies and is among the most frequently structure studied of the human musculoskeletal system over the past decades. The ACL is an intra-articular and extra synovial structure composed of numerous fascicles of dense connective tissue that connect the distal femur and the proximal tibia (Figure 1).

Two bundles of the ACL were described for the first time in 1938 by Palmer et al., followed by Abbott et al. in 1944 and Girgis et al. in 1975 [9,10,11]. Each author described an antero-medial (AM) bundle and a posterolateral (PL) bundle, named for the relative location of the tibial insertion sites. The former is the longest bundle of the anterior cruciate. The latter is characterized by the most vertical orientation within the joint. More recently, Norwood et al and Amis et al described a third bundle most similar to the AM bundle in both anatomical and biomechanical considerations [12,13]. The AM bundle attaches to the femur posteriorly and superiorly on the medial surface of the lateral femoral condyle. The insertion sites of AM and PL on the femur allows the ligament to become crossed when the knee is flexed with a horizontal alignment of femoral insertion sites of the two bundles. On the other hand, with the knee in extension, the AM and PL bundles are parallel with a vertical alignment of the femoral insertion. On the tibia, the insertions of the AM, IM and PL bundles form a triangle directed posteriorly. The AM bundle inserts on the medial aspect of the intercondylar eminence of the tibia, forming the medial corner of the triangle. The intermediate bundle attaches in the midline and lateral aspect of the eminence, lateral to the anteromedial bundle, and forms the lateral corner. The PL bundle is posterior and represent the apex of the triangle [12]. The blood supply of the anterior cruciate ligament arises from the middle geniculate artery that forms a peri-ligamentous network in the synovial sheath around the ligament.

The ACL is a unique and complex structure able to withstand multiaxial stresses and varying tensile strains thanks to its sophisticated microscopic structure. It is the primary restraint to anterior translation of the tibia relative to the femur [14]. It has been well established that the contributions of the fiber bundles to resist anterior draw forces depend on the grade of flexion of the knee (Figure 2).

Due to their anatomic insertion sites, the tension of the AM bundle increases during knee flexion. Conversely, the force in the PL bundle increases during knee extension. The ACL also functions as a major secondary restraint to internal rotation, particularly when the joint is near full extension [15].

The structural properties and tensile behavior of human ACL have been extensively studied for many decades in order to provide new baseline data for the design and selection of grafts for ACL replacement. In 1991, a landmark article published by Woo SL et al. showed the changes of the structural properties of human ACL in relation to age and orientation of the specimen. The authors found that specimens from younger subjects were characterized by higher values of stiffness and ultimate load than those from middle and older specimens. In addition, it was reported in all groups of age, that when the specimens were analyzed maintaining their anatomical angles of insertion to the femur and tibia, they were characterized by higher stiffness and ultimate load. The authors hypothesized that anatomic orienta-
tion allows a greater portion of the ACL to be loaded during tensile testing. The ultimate load for the younger specimens tested in the anatomical orientation (2160 ± 157 N) was found to be 44% higher than that of the middle-aged group (1503 ± 83 N), and 328% higher than that of the older group (658 ± 129N). On the other hand, the values of stiffness tested in anatomical orientation were 242 ± 28 N/mm, 220 ± 24 N/mm, and 180 ± 25 N/mm for the younger, middle, and older aged specimens, respectively [15].

**Fibroin silk**

Natural silk fibers are produced by arthropods such as silkworms and spiders. It has been extensively used as clinical suture material for centuries and nowadays it has been largely studied as compatible biomaterials [16]. Through different treatments, silk can be arranged to hold a broad range of forms, such as solution, powder, fibers, films, hydrogels, and sponges; this allows its use for a wide and varied use in tissue engineering. Indeed, several researches pointed out applications of silk in regeneration of bone, vascular, neural, skin, cartilage, ligament, cardiac, ocular, and bladder tissues with its advantages and limitations [17]. Silk is composed of two major proteins: Silk Fibroin (SF) and sericin. The glue-like sericin protein wraps around fibroin; it is generally soluble and can be removed by a thermo-chemical treatment. Silk fibroin is a fibrous protein constituted by a heavy (H) chain (~390 kDa) and a light (L) chain (~26 kDa) linked together via a single disulfide bond. While fibroin is responsible for the structural properties of silk, the presence of sericin renders native silk non-biocompatible. Indeed, it is able to stimulate an immune response from the host. Therefore, the chemical or enzymatic removal of sericin through a process called degum- ming is necessary [18]. The mechanical properties changed remarkably after sericin extraction. For these reasons, any biomechanical characterization of a silk scaffold should be performed using sericin extracted silk [19]. On the other hand, it seems that steam sterilization of the silk might not significantly modify the mechanical properties of silk scaffolds [20]. While the use of silk-based grafts for ACL reconstruction seems promising, the most suitable silk ACL scaffold design remain to be determined [21]. Indeed, the manner in which the silk fibroin is structured can largely affect the biomechanical performance and the survival of the graft [22]. Regarding silk ACL scaffold, several designs have been proposed to restore full knee biomechanics. Wire, braided, straight fibered and 6-wire cord represent the most common architectures explored as silk ACL scaffold. Xiang L et al. [20] and Altman et al. [19] investigated the significance differences in terms of Ultimate Tensile Strength (UTS), linear stiffness and construct elongation rate between native ACL and graft architectures. Wire, braided, straight fibered are characterized by the same size (30 mm) and total number (3456) of fibers to facilitate comparison between the several designs. 6-wire cord scaffold is constituted by 3240 fibers. Their results are reported in Table 1 [20]. We reported the results of fully hydrated yarn because it has been well established that wet condition more closely mimic the in vivo mechanic properties of native ACL [23]. The geometries of the different hierarchical architectures are described using a labeling convention of A(a)*B(b)*C(c)*D(d), where A, B, C, D represent increasing hierarchical level: Fibers (A), bundles (B), yarns (C) and cords (D), while a, b, c, d is the twisting level corresponding to the length (mm) per turn on each of the hierarchical levels.

**Table 1**: The Biomechanic characteristics of several silk ACL design.

<table>
<thead>
<tr>
<th>Ultimate tensile strength (N)</th>
<th>Static condition</th>
<th>Low load (250 cycles)</th>
<th>High load (100000 cycles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Braided silk 6 (0)*2 (2) *96 (10)*3 (12)</td>
<td>1500</td>
<td>765</td>
<td>375</td>
</tr>
<tr>
<td>Wired silk 6 (0)*2 (2) *144 (10)*2 (12)</td>
<td>1500</td>
<td>885</td>
<td>510</td>
</tr>
<tr>
<td>Straight silk 6(0)<em>2(2)</em> 288(10)*1(0)</td>
<td>1400</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wire rope (19) 30(0)*6(2)*3(2) 6(0)</td>
<td>2337 ± 72</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Stiffness (N/mm)**

<table>
<thead>
<tr>
<th>Static condition</th>
<th>Low load (250 cycles)</th>
<th>High load (100000 cycles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Braided silk 6 (0)*2 (2) *96 (10)*3 (12)</td>
<td>250</td>
<td>535</td>
</tr>
<tr>
<td>Wired silk 6 (0)*2 (2) *144 (10)*2 (12)</td>
<td>250</td>
<td>370</td>
</tr>
<tr>
<td>Straight silk 6(0)<em>2(2)</em> 288(10)*1(0)</td>
<td>900</td>
<td></td>
</tr>
<tr>
<td>Wire rope (19) 30(0)*6(2)*3(2) 6(0)</td>
<td>354 ± 26</td>
<td></td>
</tr>
</tbody>
</table>

**Elongation (mm)**

<table>
<thead>
<tr>
<th>Low load (250 cycles)</th>
<th>High load (100000 cycles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Braided silk 6 (0)*2 (2) *96 (10)*3 (12)</td>
<td>1</td>
</tr>
<tr>
<td>Wired silk 6 (0)*2 (2) *144 (10)*2 (12)</td>
<td>2</td>
</tr>
<tr>
<td>Straight silk 6(0)<em>2(2)</em> 288(10)*1(0)</td>
<td></td>
</tr>
<tr>
<td>Wire rope (19) 30(0)*6(2)*3(2) 6(0)</td>
<td>4</td>
</tr>
</tbody>
</table>
Many in vitro studies have been performed in order to evaluate the effects of surface treatments, biological factors, and cell types on silk ACL scaffold [24-30].

From literature review, it has been reported that silk scaffold combined with Mesenchymal stem cells (MSCs) was characterized by more similar biomechanical characteristics as the native ACL, making the aligned hybrid SF scaffold suitable for functional repair and regeneration of the ligament tissue [24,25]. Regarding Growth factors, it has been demonstrated that “bioactive” silk scaffold combined with stromal cell-derived factor-1 alpha or bFGF is able to improve tendon regeneration, increasing the recruitment of fibroblast-like cells, the tendon extracellular matrix production, and decreasing accumulation of inflammatory cells [26,27].

**Study in vivo**

**Table 2**

<table>
<thead>
<tr>
<th>Silk ACL design</th>
<th>Animal</th>
<th>Follow up</th>
<th>Author and Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>SeriACL scaffold</td>
<td>Goat</td>
<td>12 months</td>
<td>Horan et al. 2007 [31]</td>
</tr>
<tr>
<td>SeriACL scaffold</td>
<td>Goat</td>
<td>12 months</td>
<td>Altman et al. 2008 [32]</td>
</tr>
<tr>
<td>Braided silk cord + mesh</td>
<td>Pig</td>
<td>6 months</td>
<td>Fan et al. 2009 [33]</td>
</tr>
<tr>
<td>Knitted Silk mesh</td>
<td>Rabbit</td>
<td>4 months</td>
<td>Bi F et al. 2015 [34]</td>
</tr>
<tr>
<td>Wire - rope</td>
<td>Sheep</td>
<td>12 months</td>
<td>Teuschl et al. 2016 [35]</td>
</tr>
<tr>
<td>knitted silk mesh</td>
<td>Rabbit</td>
<td>3 months</td>
<td>Ran et al. 2017 [36]</td>
</tr>
</tbody>
</table>

In the literature, silk-based ligament grafts have been tested in animal models in only few studies. Furthermore, previous ACL studies with synthetic materials have demonstrated that the extrapolation of findings from animal data to humans needs large animal studies, like goat, sheep or pig models. To the best of our knowledge, only six studies have already tested silk-based ACL grafts in large animal studies with promising results [31-36]. Indeed, Altman et al [32] pointed out initial positive clinical, histologic, and mechanical results from a 12-month in vivo goat study demonstrating the potential of bioengineered ligament devices as clinical applications. In addition, in a pig model, Fan et al. [33] reported that their silk ligament scaffold seeded with MSCs was able to induce ligament regeneration after the 24-week post implantation period. The authors concluded that ACL scaffolds fabricated from silk fibroin might have great potential for the translation into clinical practice. Pi et al. [34] compared silk ACL scaffold with autograft in rabbit model. They reported better mechanical properties in terms of ultimate tensile load and stiffness in scaffold group than autograft. Consequently, in a sheep model, Teuschl A. et al. [35] compared a wire rope silk scaffold alone and a cell-seeded scaffold in ACL reconstruction. The authors reported that their novel silk fiber–based scaffold was able to initiate ACL regeneration under in vivo conditions, with immediate full weight-bearing and without immobilization. In addition, cell-seeded scaffold was characterized by an increased tissue regeneration at 6 months, even though difference was not observed at 12 months. Ran et al. [36] demonstrated that the silk ACL scaffold might provide sufficient vascularity and cellularity during the early stages of healing, and subsequently promote ACL regeneration and ligament-bone healing. Lastly, Horal et al. in their study implanted in 43 goats, the new hydrophilic silk scaffold named SeriACL and after 3, 6 and 12 months, they demonstrated the safety and the efficacy of the device in the animal model [31,37]. For these reasons, clinical trial with silk-based ACL grafts is currently undertaking [38].

**Discussion and future prospective**

Currently, auto grafts are the gold standard in ACL replacement. However, they do not guarantee a complete restore of the pre-injury activity levels. Furthermore, following their use, several drawbacks have been observed as morbidity, long rehabilitation period and pain of the harvest-site [39]. For these reasons, in the last thirty years of 20th century, there is a growing interest in the use of synthetic devices in ACL reconstruction because they can ensure a fast recovery and quick return to sports. Synthetic and nonabsorbable materials have been investigated for ACL replacement, including for example, Dacron® and Gore-Tex® prostheses, polypropylene-based Kennedy Ligament-Augmentation Device, carbon fiber device, Leeds-Keio Artificial Ligament and LARS ligaments (Ligament Advanced Reinforcement System) [40]. Unfortunately, even though many experimental studies have been made and much effort has been put forth, several drawbacks as mechanical breakage, plastic deformation, poor abrasion resistance, debris, lack of tissue regeneration, and stress-shielding have been some of the reasons of their withdrawal or abundance in clinical practice [40]. For these reasons, the researches started to consider the need of a bioengineered scaffold as ACL regeneration. The ideal scaffold should mimic the Extra Cellular Matrix (ECM) of the native ligament in order to provide appropriate mechanical support and biochemical stimulation. It needs to be able to manipulate cell behaviors inducing functions as proliferation, differentiation cells and matrix remodeling. Furthermore, the mechanical characteristics should be as similar as possible to those of the natural ligament. Hence, a bioengineered scaffold for ACL needs to have the ability to regenerate and remodel ingrown tissue while transferring the load-bearing from the implanted device to newly developed tissue. From a mechanic point of view, when considering a material for use as an ACL replacement, the entire stress–strain curve must be considered in its design. For example, the ultimate tensile strenght at which the graft enters into permanent plastic deformation, needs to be similar to the native ACL because if it was lesser, physiological loading regimes normally sustained by a functional ACL might permanently deform the graft (Figure 3).
Silk has several major advantages over other protein-based biomaterials, which are derived from tissues of allogeneic or xenogeneic origins. Firstly, the risk of infection is higher using those materials. Secondly, the use of silk for biomedical applications, it is economically advantageous because it is available in large scale through infrastructures for textile industries [16]. Thirdly, the constructed tissue interacts with the body’s immune system without any adverse effect. Lastly, the morphology, mechanical modulus, and degradation rate of silk-based materials are easily controlled, which makes fibroin an impressive polymeric biomaterial suitable for ligament repair. The major advantage of silk compared to other natural biopolymers is its excellent mechanical property. Silk offers an attractive balance of modulus, breaking strength, and elongation, which contributes to its good toughness and ductility. The strength-to-density ratio of silk is up to ten times higher than that of steel. Fibroin has high extensibility and exhibit marked strain hardening behavior. Such behavior is particularly important for energy absorbing scaffold [17]. Furthermore, compared to other scaffolds as PLGA, silk derived from Bombyx mori possesses a slower degradation rate, permitting a gradual transfer of load from silk-based scaffolds to the healing ligament/tendon over a period of 6–12 months [34]. We hypothesize that by providing a structural scaffold which anticipates ACL repair mechanisms, an “engineered” autologous ligament with excellent functional integrity can be developed by the body itself. Until now, as indicated by the publication data, most studies of silk-based biomaterials are focused on repairing ligament. However, despite the progress made, their clinical applications are still scarce. Surely, the animal models as sheep, goat or pig have the common limitations that all animal models of ACL surgery have. Human conditions are never fully represented in a quadruped, and post-operative rehabilitation is difficult to control in any animal model. In addition, in spite of these encouraging results, concerns on long term safety of silk materials in vivo still remain to be eliminated. Primarily, as silk materials for tissue engineering are in close contact with tissues for an extended time period, the long-term response of innate and adaptive immune system based on the type of construct and the location of implant site requires further investigations. For these reasons, based on the promising animal studies, pilot studies on human are necessary in order to fully evaluate the biological performance of the developed silk scaffolds.

References


