OSTEOARTHRITIS

MEDDOCS
International

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

L Farinelli[1]*; Y Ling-wei[1]; S Manzotti[1]; E Senigagliesi[1]; G Filippone[1]; A Aquili[1]; A Gigante[2]

[1]*Clinical Orthopaedics, Department of Clinical and Molecular Sciences, Ancona, Italy*

[2]*Gansu Provincial Hospital of Traditional Chinese Medicine, Lanzhou 730050, China*

Corresponding Author: L Farinelli

Clinical Orthopaedics, Department of Clinical and Molecular Sciences, Ancona, Italy

Email: farinelli.luca92@gmail.com

Published Online: May 22, 2019

eBook: Osteoarthritis

Publisher: MedDocs Publishers LLC

Online edition: http://meddocsonline.org/

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).

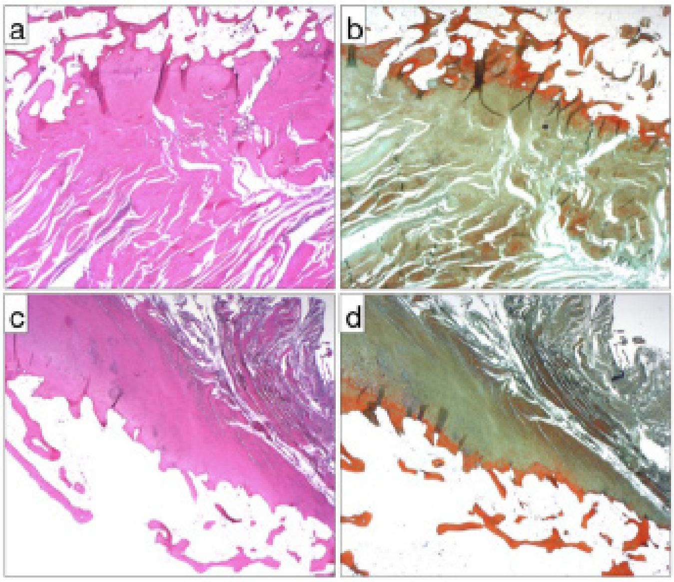

Figure 1: The insertion of human ACL in femur (a,b) and tibia (c,d). b and d: Enthesis stained with paraldeide fucsina gomori, 2,5X of magnification; a and c: Enthesis stained with hematoxylin eosin (EE), 2, 5X of magnification

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 anteromedial (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 of the ACL anatomy known as the intermediate (IM) bundle that is 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 PL bundle attaches anteriorly and inferiorly on the lateral 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).

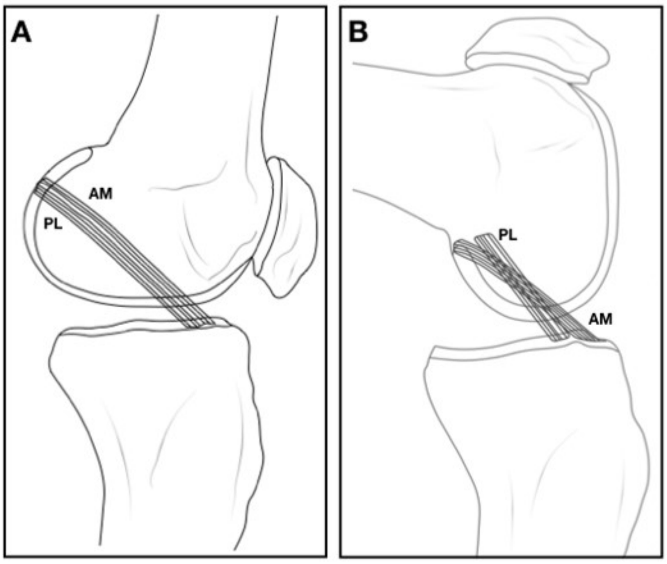

Figure 2: Schematic representation of ACL bundles in full knee extension (A) and 90° flexion (B).

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 orientation 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 anatomic 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 degumming 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 caracteristics of several silk ACL design.

		Braided silk 6 (0)*2 (2) *96 (10)*3 (12)	**Wired silk** 6 (0)*2 (2)* 144 (10)*2 (12)	**Straight silk** 6(0)*2(2)* 288(10)*1(0)	**Wire rope** (19) 30(0)*6(2)*3(2) 6(0)	**Native ACL** (15)
Ultimate tensile strength (N)	Static condition	1500	1500	1400	2337 ± 72	**2160 ± 157**
	Low load (250 cycles)	765	885			
	High load (100000 cycles)	375	510			
Stiffness (N/ mm)	Static condition	250	250	900	354 ± 26	**242 ± 28**
	Low load (250 cycles)	535	370			
	High load (100000 cycles)	570	425			
Elongation (mm)	Low load (250 cycles)	1	2			
	High load (100000 cycles)	4	4			

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

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

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).

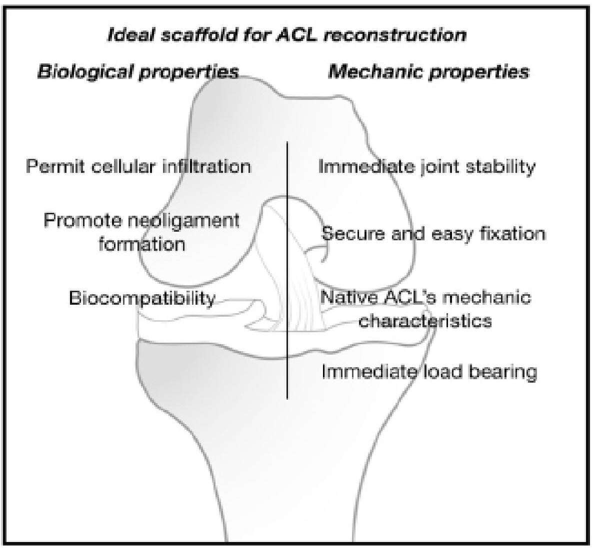

Figure 3: The ideal Scaffold for ACL regeneration

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

1. Levine JW, Kiapour AM, Quatman CE, Wordeman SC, Goel VK, et al. Clinically relevant injury patterns after an anterior cruciate ligament injury provide insight into injury mechanisms. Am J Sports Med. 2012; 41: 385-95.
2. Hewett TE, Di Stasi SL, Myer GD. Current concepts for injury prevention in athletes after anterior cruciate ligament reconstruction. Am J Sports Med. 2013; 41: 216–224.
3. Ruptured crucial ligaments and their repair by operation. AW, Robson. Ann Surg. 1903; 37: 716–718.
4. Kiapour AM, Murray MM. Basic science of anterior cruciate ligament injury and repair. Bone Joint Res. 2014; 3: 20-31.
5. Hoshino Y, Fu FH, Irrgang JJ, Tashman S. Can joint contact dynamics be restored by anterior cruciate ligament reconstruction?. Clin Orthop Relat Res. 2013; 471: 2924– 2931.
6. Leong NL, Petrigliano FA, McAllister DR. Current tissue engineering strategies in anterior cruciate ligament reconstruction. J Biomed Mater Res A. 2014; 102: 1614-1624.
7. Longo UG, Rizzello G, Berton A, et al. Synthetic grafts for anterior cruciate ligament reconstruction. Curr Stem Cell Res Ther. 8; 429-437.
8. Farè S, Torricelli P, Giavaresi G, Bertoldi S, Alessandrino A, et al. In vitro study on silk fibroin textile structure for anterior cruciate ligament regeneration. Mater Sci Eng C Mater Biol Appl. 2013; 33: 3601-8.
9. Palmer. On the injuries to the ligaments of the knee joint. Acta Chir Scand. 1938; 282.
10. Abbott LC, Saunders JB, Bost FC, et al. J Bone Joint Surg Am.Injuries to the ligaments of the knee joint. 1994; 503–521.
11. Girgis FG, Marshall JL, Al Monajem ARS. The cruciate ligaments of the knee joint. Clinic Orthop. 1975; 216–231.
12. Norwood LA, Cross MJ. Anterior cruciate ligament: Functional anatomy of its bundles in rotary instabilities. Am J Sports Med. 1979; 23.
13. Amis AA, Dawkins GPC. Functional anatomy of the anterior cruciate ligament. Fibre bundle actions related to ligament replacement and injuries. J Bone Joint Surg Br. 1991; 260–267.
14. Butler DL, Noyes FR, Grood ES. Ligamentous restraints to anterior– posterior drawer in the human knee. J Bone Joint Surg. 1980; 259.
15. Woo SL-Y, Hollis M, Adams DJ, et al. Tensile properties of the human femur-anterior cruciate ligament–tibia complex. The effects of specimen age and orientation. Am J Sports Med. 1991. 217–225.
16. Osnat Hakimi, David P. Knight, Fritz Vollrath, Pankaj Vadgama. Spider and mulberry silkworm silks as compatible biomaterials. Composites Part B: Engineering. 2007; 38: 324-337.
17. Kundu B, Rajkhowa R, Kundu SC, Wang X. Silk fibroin biomaterials for tissue regenerations. Adv Drug Deliv Rev. 2013; 65: 457-470.
18. Panilaitis B, Altman GH, Chen J, Jin HJ, Karageorgiou V, Kaplan DL. Macrophage responses to silk. Biomaterials. 2003; 3079–85.
19. Silk matrix for tissue engineered anterior cruciate ligaments. Altman GH, Horan RL, Lu HH, Moreau J, Martin I, Richmond JC, Kaplan DL. 20, 2002, Biomaterials. 2002; 23: 4131-41.
20. Li X, Snedeker JG. Wired silk architectures provide a biomimetic ACL tissue engineering scaffold. J Mech Behav Biomed Mater. 2013; 30-40.
21. Defining a scaffold for ligament tissue engineering: What has been done, and what still needs to be done. Cédric Laurent, Xing Liu, Natalia De Isla, Xiong Wang, Rachid Rahouadj. 1, 2018, Journal of Cellular Immunotherapy. 2018; 4: 4-9.
22. Fabrication and Characterization of Biphasic Silk Fibroin Scaffolds for Tendon/Ligament-to-Bone Tissue Engineering. Font Tellado S, Bonani W, Balmayor ER, Foehr P, Motta A, Migliaresi C, van Griensven M. Tissue Eng Part A. 2017; 23: 859-872.
23. Horan RL, Collette AL, Lee C, Antle K, Chen J, Altman GH. Yarn design for functional tissue engineering. J Biomech. 2006; 39: 2232-40.
24. Teh TKH, Toh SL, Goh JCH, et al. Aligned hybrid silk scaffold for enhanced differentiation of nesenchymal stem cells into ligament fibroblasts. Tissue Engineering Part C-Methods. 2011; 17. 687–703.
25. Liu H, Fan H, Wang Y, Toh SL, Goh JC. The interaction between a combined knitted silk scaffold and microporous silk sponge with human mesenchymal stem cells for ligament tissue engineering. Biomaterials. 2008; 29: 662-74.

26. Shen W, Chen X, Chen J, Yin Z, Heng BC, Chen W, Ouyang HW. The effect of incorporation of exogenous stromal cell-derived factor-1 alpha within a knitted silk- collagen sponge scaffold on tendon regeneration. Biomaterials. 2010; 31: 7239–7249.

27. Sahoo S, Toh SL, Goh JC. A bFGF-releasing silk/PLGA-based biohybrid scaffold for ligament/tendon tissue engineering using mesenchymal progenitor cells. Biomaterials. 2010; 31: 2990-8.

28. Wang X, Qiu Y, Carr AJ, Triffitt JT, Sabokbar A, Xia Z. Improved human tenocyte proliferation and differentiation in vitro by optimized silk degumming. Biomed Mater. 2011; 6: 035010.

29. He P, Sahoo S, Ng KS, Chen K, Toh SL, Goh JC. Enhanced osteoinductivity and osteoconductivity through hydroxyapatite coating of silk-based tissue-engineered ligament scaffold. J Biomed Mater Res A. 2013; 101: 555-66.

30. Chen K, Sahoo S, He P, Ng KS, Toh SL, Goh JC. A hybrid silk/RADA-based fibrous scaffold with triple hierarchy for ligament regeneration. Tissue Eng Part A. 2012; 18: 1399-409.

31. Horan RL, Richmond JC, Weitzel PP, et al. Calgary, Alberta, Canada; Clinical, mechanical and histopathological evaluation of a bioengineered long-term bioresorbable silk fibroin graft in a one year goat study for development of a functional autologous anterior cruciate ligament. Paper presented at: 2007 Annual Meeting of the American Orthopaedic Society for Sports Medicine. 2007.

32. Altman GH, Horan RL, Weitzel P, Richmond JC. The use of long-term bioresorbable scaffolds for anterior cruciate ligament repair. J Am Acad Orthop Surg. 2008; 177-187.

33. Fan H, Liu H, Toh SL, Goh JC. Anterior cruciate ligament regeneration using mesenchymal stem cells and silk scaffold in large animal model. Biomaterials. 2009; 4967-4977.

34. Bi F, Shi Z, Liu A, Guo P, Yan S. Anterior Cruciate Ligament Reconstruction in a Rabbit Model Using Silk-Collagen Scaffold and Comparison with Autograft. PLoS ONE. 2015; 10.

35. Teuschl A, Heimel P, Nürnberger S, van Griensven M, Redl H, Nau T. A Novel Silk Fiber-Based Scaffold for Regeneration of the Anterior Cruciate Ligament: Histological Results From a Study in Sheep. Am J Sports Med. 2016; 44: 1547-57.

36. Jisheng Ran, Yejun Hu, Huihui Le. Ectopic tissue engineered ligament with silk collagen scaffold for ACL regeneration: A preliminary study. Acta Biomaterialia. 2017; 307–317.

37. Horan RL, Toponarski I, Boepple HE, Weitzel PP, Richmond JC, et al. Design and characterization of a scaffold for anterior cruciate ligament engineering. J Knee Surg. 2019; 22: 82-92.

38. I, Serica Technologies. SeriACLTM Device (Gen IB) Trial for Anterior Cruciate Ligament (ACL) Repair (NCT00775892). 2008.

39. Anoka, Natasha & Nyland, John & McGinnis, Mark & Dave, Lee & Doral, Mahmut & Caborn, David. Consideration of growth factors and bio-scaffolds for treatment of combined grade II MCL and ACL injury. Knee surgery, sports traumatology, arthroscopy. 2011; 878-888.

40. Legnani C, Ventura A, Terzaghi C, Borgo E, Albisetti W. Anterior cruciate ligament reconstruction with synthetic grafts. A review of literature. Int Orthop. 2010; 34: 465-71.