Minireview

Silk scaffolds for musculoskeletal tissue engineering

Danyu Yao[1], Haifeng Liu[1] and Yubo Fan[1,2]

[1]Key Laboratory for Biomechanics and Mechanobiology of Ministry of Education, School of Biological Science and Medical Engineering, Beihang University, Beijing 100191, People's Republic of China; [2]National Research Center for Rehabilitation Technical Aids, Beijing 100176, People's Republic of China

Corresponding authors: Haifeng Liu. Email: haifengliu@buaa.edu.cn; Yubo Fan. Email: yubofan@buaa.edu.cn

Abstract

The musculoskeletal system, which includes bone, cartilage, tendon/ligament, and skeletal muscle, is becoming the targets for tissue engineering because of the high need for their repair and regeneration. Numerous factors would affect the use of musculoskeletal tissue engineering for tissue regeneration ranging from cells used for scaffold seeding to the manufacture and structures of materials. The essential function of the scaffolds is to convey growth factors as well as cells to the target site to aid the regeneration of the injury. Among the variety of biomaterials used in scaffold engineering, silk fibroin is recognized as an ideal material for its impressive cytocompatibility, slow biodegradability, and excellent mechanical properties. The current review describes the advances made in the fabrication of silk fibroin scaffolds with different forms such as films, particles, electrospun fibers, hydrogels, three-dimensional porous scaffolds, and their applications in the regeneration of musculoskeletal tissues.

Keywords: Silk fibroin, scaffold forms, musculoskeletal tissue engineering

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Introduction

The organ damage or function failure is a big problem that threatens human health. Millions of patients die of the diseases of organs every year. Musculoskeletal tissues, include bone, cartilage, tendon, ligament, and skeletal muscle that are easily injured or affected. However, their poor recovery ability often causes pains, joint instabilities, and even disabilities. In clinical, it is an effective way to replace the damaged parts with normal tissue or organs. The key problem we are facing is the shortage of donor. This persistent actuality spurred the development of tissue engineering.[1] As its imperious demands, most studies are focused on the musculoskeletal system.[2]

A biomaterial scaffold with appropriate surface and connected porous structure is critical for musculoskeletal tissue engineering (MTE), because cell attachment, growth, and differentiation on the materials need sufficient nutrition and oxygen to perform a desired tissue/organ function.[3] Besides, appropriate mechanical properties that match the repaired tissue are necessary.[4,5] At the same time of tissue growth, the scaffolds will gradually degrade and eventually be replaced by the new tissues. Both natural and synthetic polymers have been widely used for MTE.[6,7] Synthetic materials such as polylactic acid (PLA), polyurethane (PU), poly(lactide-co-glycolide), and polycaprolactones are very useful biomaterials due to their properties (e.g. degradation time, plasticity, and mechanical characteristics) and can be easily customized for some specific applications. Nevertheless, these advantages are eclipsed by their degradation products that are acidic and harmful to the body, which limits their potential use. In comparison with synthetic polymers, although natural biopolymers such as collagen, elastin, gelatin, and chitosan offer better cyto- and biocompatibility for the existence of embedded structural and functional molecules, they have limitations such as difficulty in processing and often with poor mechanical properties.[7]

Silk has been used as sutures in clinical for decades. The generally used silk is a member of the Bombycidae family, Bombyx Mori silk, which is known as mulberry silk. Silk is composed of two fibroin fibers with a triangle cross section that is bonded by glue-like sericin proteins.[8,9] The silk fibroin (SF) is the main component of silk. The sericin-free fiber was found to exhibit remarkable biocompatibility.[10–12] SF-based scaffolds have good biodegradation properties and their degradation products can be absorbed. Besides, there are abundant amino groups and carboxylic groups on the surface of SF which may be benefit for functional modification. SF is deemed as the most preferred natural material for use in scaffolds because its mechanical properties surpass many other biological materials. The secondary structure of SF is demonstrated to determine many of its biomaterial properties, such as mechanical properties and

biodegradability. As a result, the properties of SF scaffolds can be regulated by adjusting its secondary structure.[8–12] Among the SF scaffolds mentioned above, all of them except salt-leached processing ones have a random coil structure which is soluble in water.[8,9] After treated with alcohols or water annealing, the conformation of SF changes from the random coil to the water insoluble β-sheet or α-helix structure.[13] On the other hand, to enhance a specific property of the scaffold for certain application, surface treatment approach is developed. Materials treated with lectin,[6] plasma,[14] gelatin,[15] peptide,[16] etc. were proved to benefit the regeneration of musculoskeletal system. Surface modification has become an important protocol for MTE. Moreover, studies on other sources of SF such as non-mulberry and spider silk have got increased attention in the recent years.[17]

The present review is focused on recent researches about silk-based materials in the area of musculoskeletal tissue regeneration. Different forms of silk scaffolds are discussed, followed by their applications in bone, cartilage, tendon/ligament, and skeletal muscle regeneration.

Morphological diversification of silk scaffolds for MTE

As residual sericin may cause bio-incompatibility problems,[2,9] SF used in MTE should be degummed thoroughly first. To prepare various materials, degummed silk is dissolved, dialyzed, and centrifuged to get fresh SF solution.[13] SF solution can be fabricated into films, particles, electrospun fibers, nets, sponges, hydrogels, and three-dimensional (3D) porous scaffolds.[14–18] They have been widely used in musculoskeletal, vascular, skin, and nerve tissue engineering. Pictures of different morphologies of silk-based biomaterials for MTE are shown in Figure 1.

Films

SF films can be casted from SF solutions,[26] as well as blending with other polymers[27] or growth factors.[28] Fabrication of silk films by a layer-by-layer technique has also reported.[29] Additionally, silk films formed by Langmuir–Blodgett and spin coating have been obtained.[30,31] As most SF remains random coil structure, the stability of

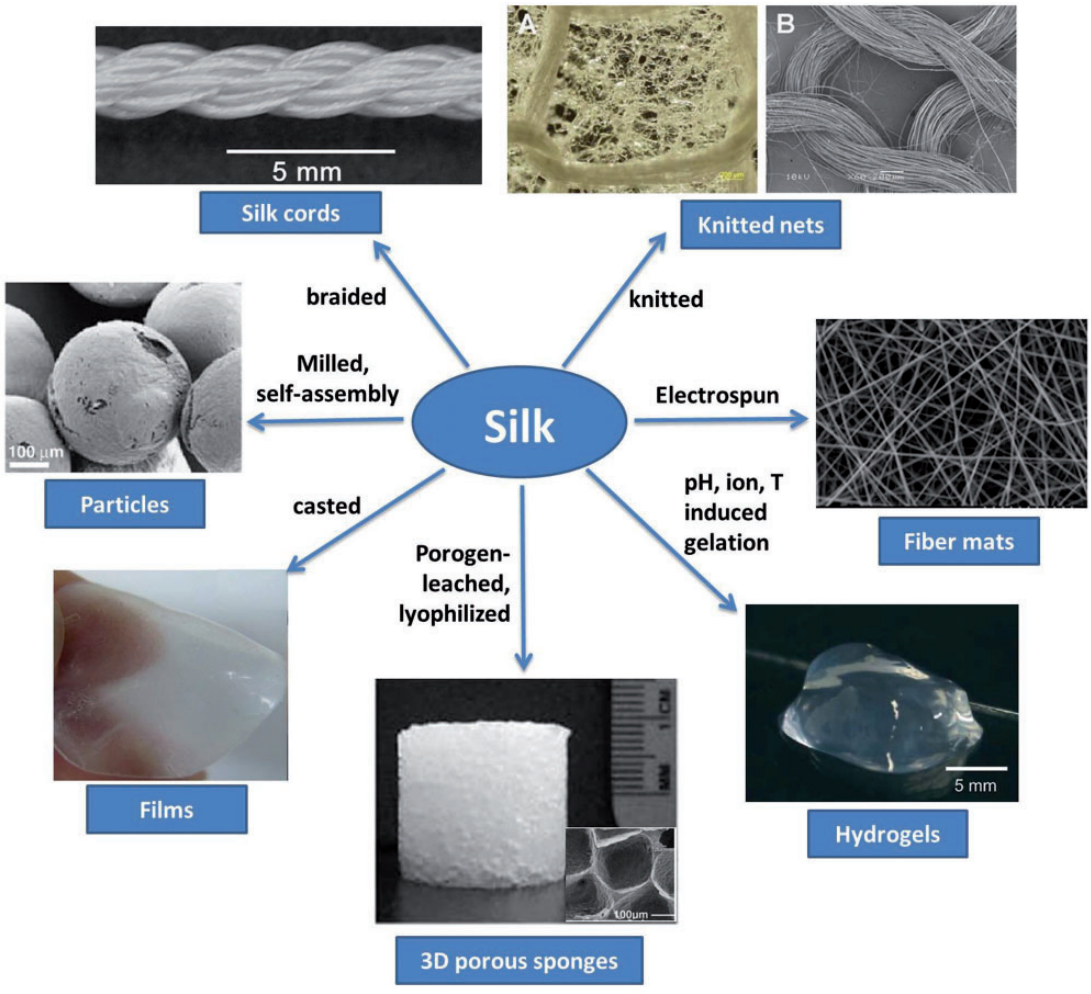

Figure 1 Representative biomaterial scaffolds fabricated from silk and silk fibroin solution for MTE: braided silk cords,[9] knitted silk nets,[19] silk fibroin films,[20] silk fibroin microparticles,[21] electrospun silk fibroin nanofibers,[22] 3D porous silk fibroin sponges,[23,24] and silk fibroin hydrogels.[25] (A color version of this figure is available in the online journal.)

such cast films is low, which leads to the water-soluble SF films. To solve this problem, the cast films should be post-treated by water annealing,[32] stretching,[33] or alcohol immersion to improve the β-sheet crystallinity. Besides, certain pretreatment of the silk solution can also obtain water-insoluble films, such as controlled drying process[26] or adding glycerinum to the SF solution.[29]

Particles

SF particles can be produced by freeze drying the SF solution or milling the solid SF into micro/nanoparticles.[34] Fabrication of silk particles by self-assembly, freeze–thawing, jet breaking, or spray drying is also reported.[21] Although milled particles are generally used for improving scaffold mechanical properties and cytocompatibilities, regenerated SF particles are mainly used for drug carriers.[35] Apart from the large specific surface and biocompatibility, the biodegradability gives silk particles a big advantage in drug delivery field. The increased significance of nanoparticles in the biomedical fields has attracted the attention of many researchers.

Electrospun fibers

Electrospun SF mats are used as biomaterials for their raised surface area. The rough feature is of benefit for cell adhesion and proliferation.[22] Appropriate viscosity of the solution is required and the solvent should be volatile to prevent the spinning fiber pasting. The all-aqueous process-produced SF electrospun mats have good biocompatibility. However, it is difficult for SF aqueous solution to meet the requirements of electrostatic spinning. Fibers have a large variation in diameter size and an irregular morphology.[36] Thus, organic solvents such as hexafluoroisopropanol (HFIP) and formic acid are widely used instead of water.[37] Organic solvents-based solution can be easily fabricated into electrospun mats with a relatively uniform fiber diameter. Nevertheless, the residual organic solvent will lower the substrate cytocompatibility. Electrospun fibers from aqueous SF solution that mixed with polymers were also setted.[38] The fibers can be prepared in a variety of diameters, which is controlled by the solution; the processing mode; the ambient parameters such as viscosity and conductivity, the field strength, flow rate, temperature, and the space between the spinning tip and the collection plate.[22,37–39] The structural stability of the electrospun fibers can be further improved by methanol treatment as well.[39]

Three-dimensional porous scaffolds

The 3D biomaterial scaffolds with high porosity are crucial for cell adhesion, growth, and migration. Both the necessary nutriment and metabolic waste are transported through the porous structure. The 3D porous scaffolds are usually prepared by porogen leaching, gas foaming, or lyophilization techniques.[23,24]

Lyophilization is an important means to fabricate SF porous materials. The structure parameters of the porous materials such as strength and elongation can be regulated by controlling freezing temperature and concentration of the SF solutions.[23,24] Moreover, aligned scaffolds can be obtained by controlling the frozen conditions.[40] Pore sizes and morphologies are decided by ice crystals whose formation depends on the freezing temperature. Usually, lower freezing temperature results in smaller pore size. The pore sizes distribute between tens and hundreds microns. Freeze-dried scaffolds have good porosity and pore structure. Aligned scaffolds can be prepared by freeze drying as well,[40] while their structural stability is poor and should be treated to improve the β-sheet crystallinity.

Solvent-based scaffolds were obtained by using salt or sugar particles as porogen. The most broadly studied is salt-leached scaffolds which use sodium chloride as porogen. The pore sizes are dependent on the porogen particles. Although it is very hard to get aligned scaffolds by salt-leached method, the salt-leached scaffolds have excellent connective structure, high porosity, and easy size-regulating pores compared to freeze-dried scaffolds.[41] Moreover, the structure of salt-leached scaffolds is stable without any treatment. These benefits make salt-leached scaffolds commonly used in MTE applications.

Hydrogels

Hydrogels are 3D polymer networks with water as the dispersion medium. Hydrogels provide an option for the delivery of cells and growth factors. Moreover, the mechanical behaviors of silk hydrogels matched the load-bearing demand for some tissue regeneration. SF hydrogels can be obtained using alcohols, acids, sonication, ions, or lyophilization,[25,42] and be formed into β-sheet structures.[20] The gelation process of SF hydrogels can be regulated by pH value of the SF solution. When the pH value is close to the isoionic point, the gelation time will be short.[43] SF gelation is also affected by SF concentration, gelation temperature, concentration of ions, and the ultrasonic power. Each of the above increased factors will shorten the time of SF gelation.[43] Recently, a new hydrogel was reported to be able to transform between solution and hydrogel several times,[44] which might be helpful for the injectable delivery system.

Combined scaffolds

Scaffolds fabricated by one method miss to match all the requirements of tissue repair. To overcome the limitation, combined scaffolds have been developed. Combination fabrication is a method that fabricates scaffolds with two or more fabrication techniques, such as porous scaffold/particles,[24] porous scaffold/membrane,[45] and hydrogel/fibers.[46] Combination fabrication can better match the tissue regeneration requirement and broaden the use of SF materials.

Applications of silk scaffolds in MTE

Biomaterials used in MTE should have homologous mechanical properties, such as similar compression modulus and appropriate degradation rate to remain the morphology and function of tissues. Scaffolds used in different tissue regenerations have to be designed, respectively, based on the special requirements. To meet these requirements,

SF-based materials are trialed, and achievements are introduced below.

Bone tissue engineering

Bone is a complex, rigid, highly vascularized architecture, and specialized connective tissue composed of 70% mineral and 30% organics by dry weight.[47] The osseous tissue includes calcified intercellular substance and osteocytes. The primary role of bone is to support the body and keep organs in the body from damages. Thus, injury or disease of bone will seriously affect the quality of life. Various porous scaffolds have been prepared and the effects used in bone regeneration have been studied. However, an ideal scaffold closely mimicking the architecture of native bone is still absent.

Owing to its excellent biocompatibility and good mechanical properties, silk has received significant attention as a bone rebuilding material.[48] SF scaffolds were proved to support the differentiation of mesenchymal stem cells (MSCs) into osteoblast *in vitro.* For example, Bini *et al.*[49] fabricated a recombined spider silk film and proved its potential to induce the differentiation of human bone marrow derived mesenchymal stem cells (hMSCs) into bone tissue with osteogenitic stimulants. The recombinant spider silk improved the hMSCs osteogenic differentiation outcomes and displayed enhanced bone-related outcomes while compared with tissue culture plastic. In another study, SF scaffolds produced using different solvents, pore sizes, and structures were seeded with human adipose-derived stem cells for bone tissue engineering. The results showed that HFIP-derived SF scaffolds with a pore size of 400–600 μm demonstrated the best bone regeneration outcomes.[50]

Porous SF scaffolds were produced by Uebersax *et al.* using two different SF sources in a water- or a solvent-based process.[51] All the scaffolds were used to repair the defects in the tibia and humerus cancellous bone in a sheep model. New bone formation was found after eight weeks of implantation. However, porous 3D SF scaffolds, or SF hydrogels, usually fail to match the mechanical demands of bone tissue engineering. To generate a scaffold with satisfactory mechanical performance for osteogenic tissue formation, Rockwood *et al.* reinforced SF hydrogels with SF microparticles.[52] Compared to SF hydrogels, the compressive modulus of the reinforced scaffolds was improved over sixfold for 1:2 (matrix:particle) reinforcement. With the increase in loading of SF microparticles, the osteogenic capabilities of hMSCs significantly increased for up to six weeks and the calcium absorption was enhanced greatly.

Hydroxyapatite (HA) has similar structure to the inorganics of bone. It has been widely used in bone engineering because of its osteoinductivity.[53] The HA/SF scaffolds have been prepared by various technologies and were found to support the MSCs differentiation toward bone tissue regeneration.[54–56] Jiang *et al.*[55] produced regenerated SF microspheres and HA was deposited onto the scaffolds using an alternate soaking process. Similarly, nano HA/SF scaffold was developed and proved its compatibility with MSCs of rats.[56] Recently, Liu *et al.* proved that adding mesoporous bioactive glass/HA nanocomposite (MGHA) into SF scaffolds would adjust the mechanical performance and surface properties of the scaffolds.[57] Results of bone formation for rat cranial bone defects *in vivo* confirmed that the compound was more beneficial in bone regeneration than pure SF. It was found that the superior bone regeneration capacity of the SF/MGHA material owed to the chemical interactions between the native tissues and grafted scaffolds, as well as the scaffold surface bioactivity.

Biomaterials incorporated with growth factors have been demonstrated to have improved repair ability. For example, Li *et al.* added the bone morphogenetic protein 2 (BMP-2) into SF solution and produced scaffolds by electrospinning for hMSC culture.[58] Results showed that the scaffolds with BMP-2 expressed more calcium deposition than those without ones. Similarly, while further combined with nanoparticles of HAP, improved mineralization was observed as well. Demineralized bone matrix (DBM), a biomaterial mainly formed with BMPs and collagen, is usually used in bone regeneration for its osteoinductivity. A salt-leached 3D porous SF scaffold was produced to improve the characteristics of DBM powders and to enhance the adhesion, proliferation, and osteoblastic differentiation of BMSCs.[59]

Cartilage tissue engineering

Cartilage preserves the subchondral bone from high stresses in the joint. Cartilage is not so hard and stiff as bone which results in an efficient load distribution to avoid most damages.[60] Cartilage damage is usually associated with joint instability, sharp pain, and loss of articular motion.[60] However, cartilage is an avascular connective tissue and the self-healing capacity of cartilage is poor.[61] Several methods and matrixes have been used in the articular cartilage regeneration.[62] Among them, engineered tissue implants are noticeable and provide a hopeful approach to cartilage regeneration.

Gellynck *et al.* developed three different scaffolds with dragline silk fibers, Araneus diadematus egg sac and Bombyx mori cocoons, and examined their effects for chondrocyte adhesion and growth.[63] Chondrocytes adhered and proliferated on these scaffolds while producing extracellular matrix during the cultivation. Wang *et al.*[64] fabricated a highly porous SF scaffold through an aqueous process and combined it with hMSCs for *in vitro* cartilage regeneration. In the presence of inducers such as TGF-β3 and dexamethasone, the hMSCs favorably differentiated along the chondrogenic pathway within the 3D matrix. And then, they cultured human chondrocytes on the same SF scaffolds and found that the cell density was decisive for the differentiation of the chondrocytes.[65] This work diversified cell sources in the combination with SF-based scaffolds for cartilage regeneration applications. In another study, a three-layered SF meniscal material system was designed by Mandal *et al.* to mimic the native meniscus structure.[66] Human chondrocytes and fibroblasts were seeded on the scaffold in a manner similar to native tissue. Improved mechanical properties and ECM alignment with culture time showed that the

construct was a promising template for directed meniscus-like tissue growth.

Compared with other scaffold formats, the injectability, shape-conforming capacity, and the ability to evenly suspend chondrocytes and maintain their chondrogenic phenotype make hydrogels a more suitable scaffold for cartilage regeneration. Parkes *et al.*[67] developed SF hydrogels with controlled pore sizes between 10 and 40 μm which led to a comparable compressive modulus to cartilage. To imitate the construction and function of native cartilage, Yodmuang *et al.*[68] prepared silk microfiber-reinforced SF (SF-silk) hydrogel composites and evaluated its use for *in vitro* cartilage development. After 42 days of culture, the chondrocytes showed excellent cellular responses on SF-silk hydrogels. Fiber reinforcement led to the formation of more mechanically robust constructs in comparison to the control group. Moreover, chondrocyte-seeded SF-silk hydrogels generated cartilage constructs which fell in the region of equilibrium modulus of native cartilage tissues.

It is of great significance to establish stable cartilage in an inflammatory condition for arthritics as their joints have senior standards of pro-inflammatory cytokines. Kwon *et al.* found that under inflammatory conditions or noninflammatory conditions, chondrocytes growing in collagen and SF scaffolds both showed higher levels of cartilaginous genetic expression than those in PLA scaffolds.[69] They studied the effect of materials on the reactions of chondrocytes to tumor necrosis factor α and interleukin-1β-mediated inflammatory stimuli. The collagen and SF scaffolds had higher water-absorbing quality and released pro-inflammatory cytokines faster than PLA scaffolds.

Blends of SF and other materials were also prepared for cartilage restoration. Bhardwaj *et al.* constructed SF/chitosan blended materials and studied their use for cartilage regeneration. SF acts as a matrix for cell growth and chitosan has a molecular structure similar to glycosaminoglycans.[70] Similarly, Mirahmadi *et al.* prepared new hydrogels by adding degummed minced silk fibers or electrospinning mat of SF into the thermosensitive chitosan/glycerophosphate hydrogels. The mechanical properties of both hydrogels were obviously improved and the enhanced hydrogels improved the attachment, proliferation, as well as substrate production of chondrocytes.[46]

Ligament and tendon tissue engineering

Ligament and tendon are both dense connective tissues. Ligaments are cord-like tissues connecting bones to bones, and tendons are those that attach muscles to bones. They own high tensile strength which is vital in regulating the normal movement and the joint stability. Ligaments and tendons are usually impaired during sports and other furious activities which may lead to instability and abnormal joint movement.[71] However, they do not have a strong regeneration capacity and hence significant joint dysfunction may be caused. Different kinds of treatments have been applied for repairing ligament/tendon injuries, and ligament and tendon tissue engineering may offer a more efficient therapeutic method than the traditional approaches.

The perfect mechanical properties provided unique benefits to silk as an ideal ligament/tendon restoration material.[72–74] Spider silk bundles were braided into sutures by Hennecke *et al.*[74] and the sutures exhibited quite well tensile strength which will be appropriate to tendon restoration. Altman *et al.*[73] braided a silk fiber matrix which successfully matched the mechanical properties requirements of the human anterior cruciate ligaments. The real-time polymerase chain reaction (RT-PCR) evaluation of hMSCs-seeded scaffolds indicated that the expression ratio of collagen I to collagen III was 8.9:1, in line with that of the native cruciate ligaments. However, braded materials suffered problems in nutrient delivery and cell infiltration. To overcome these drawbacks, knitted scaffolds have been developed. Knitted scaffolds have sufficient internal spaces which are of benefit for communicating. Moreover, they avail the deposition of tissue matrix associated with collagen producing, which is crucial for ligament regeneration.[19,75,76] Gel systems are usually needed to seed cells on knitted scaffolds. However, as the cell–gel composite easily separate from scaffolds during motion, the gel systems were not suitable for ligament restoration in the joint. To solve this problem, Liu *et al.*[72] used porous SF sponges to cover the large pores of knitted silk scaffolds. While the knitted structure offered the scaffold excellent mechanical properties, the microporous structure of SF sponges mimicked the ECM which promoted cell attachment, proliferation, differentiation, and function. Subsequently, the scaffolds were used to rebuild the anterior cruciate ligament (ACL) in a rabbit model and a pig model, respectively.[75,76] After 24 weeks of implantation, MSCs distributed throughout the regenerated ligament with characteristic four zones being reconstructed. However, the micro-CT results indicated that nearly no mineralized tissue was formed in the inner tunnels of implants.

Implanted ligament grafts without the ligament-to-bone interface will lead to a poor integrating with the bone, which has been the main reason for ligament graft failure. To solve this problem, He *et al.*[77] established a co-culture system and showed that osteoblasts and fibroblasts could induce bMSCs differentiation into the fibrocartilage cells. To improve osteoblasts activities, the scaffold was coated with HA. After three weeks of culture, a gradualness transition from unmineralized to mineralized region formed in the co-cultured constructs, which was crucial for ligament–bone interface regeneration. In order to further enhance the bone tunnel regeneration, low-crystallinity HA-modified SF scaffolds were developed by Shi *et al.*[78] About 80% of primordial mechanical properties and 60% of bone volume were reverted after four months of restoration in a rabbit model. Remarkable amount of new bone formation was also verified by histological analysis and micro-CT.

Since the poor healing ability of native tendon, the regeneration of injured tendon remains a difficult challenge. Chen *et al.*[79] knitted a silk-collagen scaffold and evaluated its tendon restoration efficacy. Human embryonic stem cell (hESC)-derived MSCs were seeded onto the composite scaffolds and suffered mechanical stimulus *in vitro*. Distinctly expressed tendon-related gene makers and tenocyte-like morphology were observed. While used in a rat tendon injury model, the engineered tendon subject *in vivo*

mechanical stimulation showed larger collagen fibers and more regularly aligned cells which in turn led to reinforced tendon restoration *in situ*. In the subsequent study, the composite sponge scaffolds were also associated with the tendon-specific transcription factor scleraxis (SCX) and hMSC differentiated from hESCs.[80]

Skeletal muscle tissue engineering

Skeletal muscle is also called striated muscle. It refers to tissues that transform the chemical energy saved in glucose to the mechanical energy efficiently. Skeletal muscle is one of the most crucial functional parts of the organism's organs.[81] There are more than 600 skeletal muscles in the human body which can cause locomotion of the organism itself as well as movements of internal organs. Longitudinal arranged myofilaments composed the skeletal muscle fiber with actin and myosin as the major components. Traumatic injury or diseases such as amyotrophy may lead to muscle functional damage. Although many attempts have been made, skeletal muscle tissue regeneration is still a technological challenge.

SF benefits the proliferation and differentiation of myoblasts. It has been proved that the SF-modified PU scaffold can be used in hypopharyngeal tissue engineering. Shen *et al.* prepared a poly(ester-urethane) scaffold bonded with SF on the surface to improve its hydrophilicity and biocompatibility.[82] Compared with the control poly(ester-urethane) group, better proliferation and differentiation of skeletal muscle cells and human hypopharynx fibroblasts were observed which indicated lower cytotoxicity and better biocompatibility. While SF-bonded poly(ester-urethane) scaffold was planted hypodermic in the rat back, a better biocompatibility to the peripheral tissue and a faster degradation than the non-SF modified group was detected.

Conclusion and future perspectives

To achieve functionally active systems, the regenerated tissue should successfully establish interaction with immune system in the body. The materials should also be designed particularly. Silk-based materials are easily controlled on scaffold morphology, mechanical modulus, and degradation rate with different structure. Silk has been demonstrated to be a promising biomaterial for use in regenerative medicine.

Until now, most studies have been centered on *in vitro* regeneration of bone, cartilage, ligament, and tendon. Study about the regeneration of skeletal muscle tissue is obviously less than hard tissues. Silk-based biomaterials have been studied widely enough; however, their clinical uses are still scarce. We believe that *in vivo* mechanical environments such as pressures in the body should be considered while designing a material. Besides, more efforts should be paid on *in vivo* studies. The understanding of silk molecule structure and processing has advanced which gives more opportunities in the use of silk in MTE. Anyhow, we anticipate great progress in the investigations on silk-based materials and probabilities of clinical success in the near future.

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REFERENCES

1. Langer R, Vacanti J. Tissue engineering. *Science* 1993;**260**:920–26
2. Kasoju N, Bora U. Silk fibroin in tissue engineering. *Adv Healthcare Mater* 2012;**1**:393–412
3. Ma PX. Scaffolds for tissue fabrication. *Mater Today* 2004;**7**:30–40
4. Borchardt JK. Porous structures for tissue engineering: polymers. *Mater Today* 2004;**7**:26
5. Koning M, Harmsen MC, van Luyn MJ, Werker P. Current opportunities and challenges in skeletal muscle tissue engineering. *J Tissue Eng Regen Med* 2009;**3**:407–15
6. Teuschl AH, Neutsch L, Monforte X, Rünzler D, van Griensven M, Gabor F, Redl H. Enhanced cell adhesion on silk fibroin via lectin surface modification. *Act Biomater* 2014;**10**:2506–17
7. Cheung H-Y, Lau K-T, Lu T-P, Hui D. A critical review on polymer-based bio-engineered materials for scaffold development. *Compos Part B* 2007;**38**:291–300
8. Yao D, Dong S, Lu Q, Hu X, Kaplan DL, Zhang B, Zhu H. Salt-leached silk scaffolds with tunable mechanical properties. *Biomacromolecules* 2012;**13**:3723–29
9. Altman GH, Diaz F, Jakuba C, Calabro T, Horan RL, Chen J, Lu H, Richmond J, Kaplan DL. Silk-based biomaterials. *Biomaterials* 2003;**24**:401–16
10. Gong X, Liu H, Ding X, Liu M, Li X, Zheng L, Jia X, Zhou G, Zou Y, Li J. Physiological pulsatile flow culture conditions to generate functional endothelium on a sulfated silk fibroin nanofibrous scaffold. *Biomaterials* 2014;**35**:4782–91
11. Liu H, Li X, Zhou G, Fan H, Fan Y. Electrospun sulfated silk fibroin nanofibrous scaffolds for vascular tissue engineering. *Biomaterials* 2011;**32**:3784–93
12. Liu H, Li X, Niu X, Zhou G, Li P, Fan Y. Improved hemocompatibility and endothelialization of vascular grafts by covalent immobilization of sulfated silk fibroin on poly (lactic-co-glycolic acid) scaffolds. *Biomacromolecules* 2011;**12**:2914–24
13. Hu X, Shmelev K, Sun L, Gil E-S, Park S-H, Cebe P, Kaplan DL. Regulation of silk material structure by temperature-controlled water vapor annealing. *Biomacromolecules* 2011;**12**:1686–96
14. Cheon YW, Lee WJ, Baek HS, Lee YD, Park JC, Park YH, Ki CS, Chung KH, Rah DK. Enhanced chondrogenic responses of human articular chondrocytes onto silk fibroin/wool keratose scaffolds treated with microwave-induced argon plasma. *Artif Organs* 2010;**34**:384–92
15. Yang L, Yaseen M, Zhao X, Coffey P, Pan F, Wang YM, Xu H, Webster J, Lu JR. Gelatin modified ultrathin silk fibroin films for enhanced proliferation of cells. *Biomed Mater* 2015;**10**:025003

16. Bai L, Zhu L, Min S, Liu L, Cai Y, Yao J. Surface modification and properties of Bombyx mori silk fibroin films by antimicrobial peptide. *Appl Surf Sci* 2008;**254**:2988–95
17. Yun H, Oh H, Kim MK, Kwak HW, Lee JY, Um IC, Vootla SK, Lee KH. Extraction conditions of Antheraea mylitta sericin with high yields and minimum molecular weight degradation. *Int J Biol Macromol* 2013;**52**:59–65
18. Liu H, Ge Z, Wang Y, Toh SL, Sutthikhum V, Goh JC. Modification of sericin-free silk fibers for ligament tissue engineering application. *J Biomed Mater Res Part B* 2007;**82**:129–38
19. 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
20. Wang H-Y, Zhang Y-Q. Processing and characterisation of a novel electropolymerized silk fibroin hydrogel membrane. *Sci Rep* 2014;**4**:06182
21. Wenk E, Wandrey AJ, Merkle HP, Meinel L. Silk fibroin spheres as a platform for controlled drug delivery. *J Control Release* 2008;**132**:26–34
22. Shan Y-H, Peng L-H, Liu X, Chen X, Xiong J, Gao J-Q. Silk fibroin/gelatin electrospun nanofibrous dressing functionalized with astragaloside IV induces healing and anti-scar effects on burn wound. *Int J Pharm* 2015;**479**:291–301
23. Kim U-J, Park J, Joo Kim H, Wada M, Kaplan DL. Three-dimensional aqueous-derived biomaterial scaffolds from silk fibroin. *Biomaterials* 2005;**26**:2775–85
24. Rajkhowa R, Gil ES, Kluge J, Numata K, Wang L, Wang X, Kaplan DL. Reinforcing silk scaffolds with silk particles. *Macromol Biosci* 2010;**10**:599–611
25. Rammensee S, Huemmerich D, Hermanson K, Scheibel T, Bausch A. Rheological characterization of hydrogels formed by recombinantly produced spider silk. *Appl Phys A* 2006;**82**:261–4
26. Lu Q, Hu X, Wang X, Kluge JA, Lu S, Cebe P, Kaplan DL. Water-insoluble silk films with silk I structure. *Act Biomater* 2010;**6**:1380–7
27. Lv Q, Hu K, Feng Q, Cui F. Preparation of insoluble fibroin/collagen films without methanol treatment and the increase of its flexibility and cytocompatibility. *J Appl Polym Sci* 2008;**109**:1577–84
28. Lu Q, Zhang X, Hu X, Kaplan DL. Green process to prepare silk fibroin/gelatin biomaterial scaffolds. *Macromol Biosci* 2010;**10**:289–98
29. Lu S, Wang X, Lu Q, Zhang X, Kluge JA, Uppal N, Omenetto F, Kaplan DL. Insoluble and flexible silk films containing glycerol. *Biomacromolecules* 2009;**11**:143–50
30. Karageorgiou V, Meinel L, Hofmann S, Malhotra A, Volloch V, Kaplan D. Bone morphogenetic protein-2 decorated silk fibroin films induce osteogenic differentiation of human bone marrow stromal cells. *J Biomed Mater Res Part A* 2004;**71**:528–37
31. Wang X, Kim HJ, Xu P, Matsumoto A, Kaplan DL. Biomaterial coatings by stepwise deposition of silk fibroin. *Langmuir* 2005;**21**:11335–41
32. Hu Y, Zhang Q, You R, Wang L, Li M. The relationship between secondary structure and biodegradation behavior of silk fibroin scaffolds. *Adv Mater Sci Eng* 2012;185905
33. Zhang X, Reagan MR, Kaplan DL. Electrospun silk biomaterial scaffolds for regenerative medicine. *Adv Drug Delivery Rev* 2009;**61**:988–1006
34. Yoshimizu H, Asakura T. Preparation and characterization of silk fibroin powder and its application to enzyme immobilization. *J Appl Polym Sci* 1990;**40**:127–34
35. Seib FP, Jones GT, Rnjak-Kovacina J, Lin Y, Kaplan DL. pH-dependent anticancer drug release from silk nanoparticles. *Adv Healthcare Mater* 2013;**2**:1606–11
36. Chen C, Chuanbao C, Xilan M, Yin T, Hesun Z. Preparation of nonwoven mats from all-aqueous silk fibroin solution with electrospinning method. *Polymer* 2006;**47**:6322–7
37. Zarkoob S, Eby R, Reneker DH, Hudson SD, Ertley D, Adams WW. Structure and morphology of electrospun silk nanofibers. *Polymer* 2004;**45**:3973–7
38. Jin H-J, Fridrikh SV, Rutledge GC, Kaplan DL. Electrospinning Bombyx mori silk with poly (ethylene oxide). *Biomacromolecules* 2002;**3**:1233–9
39. Min B-M, Lee G, Kim SH, Nam YS, Lee TS, Park WH. Electrospinning of silk fibroin nanofibers and its effect on the adhesion and spreading of normal human keratinocytes and fibroblasts in vitro. *Biomaterials* 2004;**25**:1289–97
40. Oliveira A, Sun L, Kim H, Hu X, Rice W, Kluge J, Reis R, Kaplan D. Aligned silk-based 3-D architectures for contact guidance in tissue engineering. *Act Biomater* 2012;**8**:1530–42
41. Wang Y, Bella E, Lee CS, Migliaresi C, Pelcastre L, Schwartz Z, Boyan BD, Motta A. The synergistic effects of 3-D porous silk fibroin matrix scaffold properties and hydrodynamic environment in cartilage tissue regeneration. *Biomaterials* 2010;**31**:4672–81
42. Guziewicz N, Best A, Perez-Ramirez B, Kaplan DL. Lyophilized silk fibroin hydrogels for the sustained local delivery of therapeutic monoclonal antibodies. *Biomaterials* 2011;**32**:2642–50
43. Kim U-J, Park J, Li C, Jin H-J, Valluzzi R, Kaplan DL. Structure and properties of silk hydrogels. *Biomacromolecules* 2004;**5**:786–92
44. Bai S, Zhang X, Lu Q, Sheng W, Liu L, Dong B, Kaplan DL, Zhu H. Reversible hydrogel–solution system of silk with high beta-sheet content. *Biomacromolecules* 2014;**15**:3044–51
45. Tu DD, Chung YG, Gil ES, Seth A, Franck D, Cristofaro V, Sullivan MP, Di Vizio D, Gomez P, Adam RM. Bladder tissue regeneration using acellular bi-layer silk scaffolds in a large animal model of augmentation cystoplasty. *Biomaterials* 2013;**34**:8681–9
46. Mirahmadi F, Tafazzoli-Shadpour M, Shokrgozar MA, Bonakdar S. Enhanced mechanical properties of thermosensitive chitosan hydrogel by silk fibers for cartilage tissue engineering. *Mater Sci Eng C* 2013;**33**:4786–94
47. Kim HJ, Kim U-J, Kim HS, Li C, Wada M, Leisk GG, Kaplan DL. Bone tissue engineering with premineralized silk scaffolds. *Bone* 2008;**42**:1226–34
48. Zhang Y, Fan W, Ma Z, Wu C, Fang W, Liu G, Xiao Y. The effects of pore architecture in silk fibroin scaffolds on the growth and differentiation of mesenchymal stem cells expressing BMP7. *Act Biomater* 2010;**6**:3021–28
49. Bini E, Foo CWP, Huang J, Karageorgiou V, Kitchel B, Kaplan DL. RGD-functionalized bioengineered spider dragline silk biomaterial. *Biomacromolecules* 2006;**7**:3139–45
50. Correia C, Bhumiratana S, Yan L-P, Oliveira AL, Gimble JM, Rockwood D, Kaplan DL, Sousa RA, Reis RL, Vunjak-Novakovic G. Development of silk-based scaffolds for tissue engineering of bone from human adipose-derived stem cells. *Act Biomater* 2012;**8**:2483–92
51. Uebersax L, Apfel T, Nuss KM, Vogt R, Kim HY, Meinel L, Kaplan DL, Auer JA, Merkle HP, von Rechenberg B. Biocompatibility and osteoconduction of macroporous silk fibroin implants in cortical defects in sheep. *Eur J Pharm Biopharm* 2013;**85**:107–18
52. Rockwood DN, Gil ES, Park S-H, Kluge JA, Grayson W, Bhumiratana S, Rajkhowa R, Wang X, Kim SJ, Vunjak-Novakovic G. Ingrowth of human mesenchymal stem cells into porous silk particle reinforced silk composite scaffolds: An in vitro study. *Act Biomater* 2011;**7**:144–51
53. Barradas A, Yuan H, Blitterswijk CA, Habibovic P. Osteoinductive biomaterials: current knowledge of properties, experimental models and biological mechanisms. *Eur Cells Mater* 2011;**21**:407–29
54. Lima PAL, Resende CX, de Almeida Soares GD, Anselme K, Almeida LE. Preparation, characterization and biological test of 3D-scaffolds based on chitosan, fibroin and hydroxyapatite for bone tissue engineering. Mater Sci Eng *C* 2013;**33**:3389–95
55. Jiang J, Hao W, Li Y, Yao J, Shao Z, Li H, Yang J, Chen S. Hydroxyapatite/regenerated silk fibroin scaffold-enhanced osteoinductivity and osteoconductivity of bone marrow-derived mesenchymal stromal cells. *Biotechnol Lett* 2013;**35**:657–61
56. Tanaka T, Hirose M, Kotobuki N, Ohgushi H, Furuzono T, Sato J. Nanoscaled hydroxyapatite/silk fibroin sheets support osteogenic differentiation of rat bone marrow mesenchymal cells. *Mater Sci Eng C* 2007;**27**:817–23
57. Liu T, Ding X, Lai D, Chen Y, Zhang R, Chen J, Feng X, Chen X, Yang X, Zhao R. Enhancing in vitro bioactivity and in vivo osteogenesis of organic–inorganic nanofibrous biocomposites with novel bioceramics. *J Mater Chem B* 2014;**2**:6293–305
58. Li C, Vepari C, Jin H-J, Kim HJ, Kaplan DL. Electrospun silk-BMP-2 scaffolds for bone tissue engineering. *Biomaterials* 2006;**27**:3115–24

59. Ding X, Wei X, Huang Y, Guan C, Zou T, Wang S, Liu H, Fan Y. Delivery of demineralized bone matrix powder using a salt-leached silk fibroin carrier for bone regeneration. *J Mater Chem B* 2015;**3**:3177–88
60. Swieszkowski W, Tuan BHS, Kurzydlowski KJ, Hutmacher DW. Repair and regeneration of osteochondral defects in the articular joints. *Biomol Eng* 2007;**24**:489–95
61. Yan L-P, Oliveira JM, Oliveira AL, Caridade SG, Mano JF, Reis RL. Macro/microporous silk fibroin scaffolds with potential for articular cartilage and meniscus tissue engineering applications. *Act Biomater* 2012;**8**:289–301
62. Luo Z, Jiang L, Xu Y, Li H, Xu W, Wu S, Wang YL, Tang ZY, Lv YG, Yang L. Mechano growth factor (MGF) and transforming growth factor (TGF)-β3 functionalized silk scaffolds enhance articular hyaline cartilage regeneration in rabbit model. *Biomaterials* 2015;**52**:463–75
63. Gellynck K, Verdonk PC, Van Nimmen E, Almqvist KF, Gheysens T, Schoukens G, Van Langenhove L, Kiekens P, Mertens J, Verbruggen G. Silkworm and spider silk scaffolds for chondrocyte support. *J Mater Sci Mater Med* 2008;**19**:3399–409
64. Wang Y, Kim U-J, Blasioli DJ, Kim H-J, Kaplan DL. In vitro cartilage tissue engineering with 3D porous aqueous-derived silk scaffolds and mesenchymal stem cells. *Biomaterials* 2005;**26**:7082–94
65. Wang Y, Blasioli DJ, Kim H-J, Kim HS, Kaplan DL. Cartilage tissue engineering with silk scaffolds and human articular chondrocytes. *Biomaterials* 2006;**27**:4434–42
66. Mandal BB, Park S-H, Gil ES, Kaplan DL. Multilayered silk scaffolds for meniscus tissue engineering. *Biomaterials* 2011;**32**:639–51
67. Parkes M, Dini D, Myant C, Cann P. Tribology-optimised silk protein Hydrogels for articular cartilage repair. *Tribol Int* 2015;**89**:9–18
68. Yodmuang S, McNamara SL, Nover AB, Mandal BB, Agarwal M, Kelly T-AN, Chao P-hG, Hung C, Kaplan DL, Vunjak-Novakovic G. Silk microfiber-reinforced silk hydrogel composites for functional cartilage tissue repair. *Act Biomater* 2015;**11**:27–36
69. Kwon H, Sun L, Cairns DM, Rainbow RS, Preda RC, Kaplan DL, Zeng L. The influence of scaffold material on chondrocytes under inflammatory conditions. *Act Biomater* 2013;**9**:6563–75
70. Bhardwaj N, Nguyen QT, Chen AC, Kaplan DL, Sah RL, Kundu SC. Potential of 3-D tissue constructs engineered from bovine chondrocytes/silk fibroin-chitosan for in vitro cartilage tissue engineering. *Biomaterials* 2011;**32**:5773–81
71. Aslan H, Kimelman-Bleich N, Pelled G, Gazit D. Molecular targets for tendon neoformation. *J Clin Invest* 2008;**118**:439–44
72. Liu H, Fan H, Toh SL, Goh JC. A comparison of rabbit mesenchymal stem cells and anterior cruciate ligament fibroblasts responses on combined silk scaffolds. *Biomaterials* 2008;**29**:1443–53
73. Altman GH, Horan RL, Lu HH, Moreau J, Martin I, Richmond JC, Kaplan DL. Silk matrix for tissue engineered anterior cruciate ligaments. *Biomaterials* 2002;**23**:4131–41
74. Hennecke K, Redeker J, Kuhbier JW, Strauss S, Allmeling C, Kasper C, Reimers K, Vogt PM. Bundles of spider silk, braided into sutures, resist basic cyclic tests: potential use for flexor tendon repair. *PLoS One* 2013;**8**:e61100
75. Fan H, Liu H, Wong EJ, Toh SL, Goh JC. In vivo study of anterior cruciate ligament regeneration using mesenchymal stem cells and silk scaffold. *Biomaterials* 2008;**29**:3324–37
76. 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;**30**:4967–77
77. He P, Ng KS, Toh SL, Goh JCH. In vitro ligament–bone interface regeneration using a trilineage coculture system on a hybrid silk scaffold. *Biomacromolecules* 2012;**13**:2692–703
78. Shi P, Teh TK, Toh SL, Goh JC. Variation of the effect of calcium phosphate enhancement of implanted silk fibroin ligament bone integration. *Biomaterials* 2013;**34**:5947–57
79. Chen JL, Yin Z, Shen WL, Chen X, Heng BC, Zou XH, Ouyang HW. Efficacy of hESC-MSCs in knitted silk-collagen scaffold for tendon tissue engineering and their roles. *Biomaterials* 2010;**31**:9438–51
80. Chen X, Yin Z, Shen W-l, Fang Z, Zhu T, Ji J, Ouyang H-W, Zou X-H. Scleraxis-overexpressed human embryonic stem cell–derived mesenchymal stem cells for tendon tissue engineering with knitted silk-collagen scaffold. *Tissue Eng Part A* 2014;**20**:1583–92
81. Fujita H, Shimizu K, Nagamori E. Novel method for fabrication of skeletal muscle construct from the C2C12 myoblast cell line using serum-free medium AIM-V. *Biotechnol Bioeng* 2009;**103**:1034–41
82. Shen Z, Kang C, Chen J, Ye D, Qiu S, Guo S, Zhu Y. Surface modification of polyurethane towards promoting the ex vivo cytocompatibility and in vivo biocompatibility for hypopharyngeal tissue engineering. *J Biomater Appl* 2013;**28**:607–16

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