Fiber-based scaffolding techniques for tendon tissue engineering

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Abstract

Tendon refers to a band of tough, regularly arranged and connective tissue connecting muscle and bone, transferring strength from muscle to bone, and enabling articular stability and movement. The limitations of natural tendon grafts motivate the scaffold-based tissue engineering (TE) approaches, which aim to build patient-specific biological substitutes that can repair the damaged or diseased tissues. Advances in engineering and knowledge of chemistry and biology have brought forth numerous fiber-based technologies, including electrospinning, electrohydrodynamic jet printing, electrochemical alignment technique and other fiber-assembly technologies, which enable the fabrication of tendon tissue structure in three-dimension. Textile techniques such as knitting and braiding have also been performed based on the fibrous materials to produce more complex structure. These scaffolds showed great similarity with native tendons in architectural features, mechanical properties, and facilitate biological functionality such as cellular adhesion, ingrowth, proliferation and differentiation towards tendon tissue. Herein, we review the techniques which have been used to assemble fibers into scaffolds for tendon TE application. The morphological structures, mechanical properties, materials, degradation characteristics and biological activities of the induced scaffolds were compared. The existing challenges and future prospects of fiber-based tendon TE have also been discussed.

Keywords: tendon regeneration, biomaterials, crimped fibers, textile processing, scaffold design, cellular alignment
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1. Introduction to Human Tendons
   1.1 Tendon Injury and Current Treatments
Tendon injury occupies about 50% of the musculoskeletal injuries in the United States (Juncosa-Melvin et al., 2006), which has become one of the most popular injuries in human body, leading to more than 100,000 patients experiencing tendon-related treatment (Goulet et al., 2011). With the increased human life span, it has been predicted that tendon-related problems may happen to 25% of all adults (Longo et al., 2010). Tendons are injured mainly by single vigorous impact or frequent stretching under low magnitude forces (Bass, 2011).

Tendinitis and tendinosis are two types of tendon injuries, which are also referred to as tendinopathies (Bass, 2011). These types of injuries involve degenerative changes in the presence (tendinitis) or absence (tendinosis) of inflammatory processes (Peers and Lysens, 2005, Nirschl and Ashman, 2003). Acute ruptures of tendons are also frequently occurring tendon injuries, especially in middle-aged men (Flik et al., 2005). The commonly injured tendons include the rotator cuff in shoulder, the extensor in forearm, the flexor tendon in hand, the Achilles tendon and the tibias posterior in shank, and the anterior cruciate ligament (ACL) and the patellar tendon in knee (Roos et al., 1995). For instance, 16% of the public population and 80% of individuals older than 80 years suffer from rotator cuff injuries (Aström and Rausing, 1995), and patellar tendon injuries are frequently found in jumping sport athletes (Lomas et al., 2015).

The therapy used currently to heal tendon injuries includes surgical suture, transplants such as autograft, allograft, xenograft, and permanent tendon prostheses (Jørgensen et al., 2001). Suture involves an end-to-end repair to hold the ruptured tendon together (Rawson et al., 2013). However, suture is not applicable for tendon healing with gaps larger than 5 mm (Burkhart et al., 1997). Autograft is considered the “gold standard” for large tendon treatment owing to its advantages include absence of immunological problems and good remodelling. However, autograft is also accompanied with some disadvantages such as increased operation duration due to the time used for graft preparation, limited donor
tendon source, and sacrifice of the original function of the donated tendon. The drawbacks of autograft encourage the use of allografts (Prokopis and Schepsis, 1999, Olender et al., 2011). However, allografts are accompanied with problems including immunological rejection, disease transmission, high re-rupture rate, and mismatch of age, gender and body weight between donors and recipients. As another alternative, xenografts emerge (Chen et al., 2009, Turner and Badyak, 2013). However, the pre-processing such as decellularisation and sterilisation, performed to prevent immune rejection leads to the decrease of original mechanical strength, and thus results in high failure rates for both allografts (Noyes and Barber-Westin, 1996, Marralle et al., 2007) and xenografts (Galili, 2012). Permanent prostheses/artificial tendons fabricated using non-biodegradable materials such as polyethylene (Grau, 1958), Teflon (Gonzalez, 1959) and silicon (Hunter, 1965), have also been reported. In these cases, the artificial tendons lack the capacity of mimicking organization of the original tendons. In addition, wear debris and degraded by-products result in immunological reactions and acidifications in surrounding tissue.

1.2 Structure, Composition and Mechanical Properties of Human Tendons

Tendon is a bundle of fibrous, regularly organized and connective tissue interposed between muscle and bone (Doroski et al., 2007). In different parts of body, the shapes of tendons diverge significantly including cylindrical, wide and flat, fan-shaped and ribbon-shaped tendons (Józsa and Kannus, 1997). As critical components of the joints, tendons possess various important functions including transferring loads between muscles and bones, and making joint stability and movement possible (Jozsa et al., 1991). A tendon is primarily comprised of collagen (Lomas et al., 2015). The collagen molecules with a triple helical structure link end-to-end to form a collagen fibril (e.g. 20-150 nm in diameter in human tendons). A collagen fiber, the fundamental component of a tendon, forms by a bunch of
collagen fibrils and varies significantly in different tendon types. For example, collagen fiber of extensor pollicis longus tendon in thumb, biceps brachii in arm, quadriceps tendon in thigh, Achilles tendon in ankle and patellar tendon in knee are about 19, 20, 22, 26 and 17 μm in diameter, respectively (Józsa and Kannus, 1997, Yahia and Drouin, 1989). A tendon has a well-arranged structure with different levels of fibrous bundles, namely the primary fiber bundle (i.e. subfascicle), secondary fiber bundle (i.e. fascicle) and tertiary bundles (Figure 1). The diameters of subfascicles and fascicles depend on the entire tendon size. Thus, small tendons (e.g. flexors and extensors in fingers and toes) have thinner subfascicles and fascicles whilst big tendons (e.g. Achilles tendon) have thicker ones (Józsa and Kannus, 1997). In human, the fascicles range from 150 to 1000 μm in diameter, and the tertiary bundles vary from 1 to 3 mm in diameter.

As for tendon composition, human tendon tissues are comprised of collagens, elastin, proteoglycans (PGs) and glycosaminoglycans (GAGs), with slightly different concentration among different tendon types (Lomas et al., 2015). The dry mass of tendon composes approximately 80-90 % collagen type I (COL-I), 1-2 % elastin, 1-5 % PGs and 0.2 % GAGs (Kannus, 2000), and water constitutes about 60-80 % of the tendon wet weight (Hess et al., 1989). In tendons, COL-I dominates, while small amounts of other types of collagens also exist, which are remarkably related to tendon aging and pathophysiology (Wenstrup et al., 2011). Apart from the extracellular components, tendons also contain various cell populations (Kannus, 2000). Tenoblasts and their mature form, termed tenocytes, occupy 90-95 % of the cells in tendons. Particularly, a rare population of tendon stem/progenitor cells (hTSPCs) has been isolated and identified from human and mouse, which exhibit universal stem cell criteria such as clonogenicity, self-renewal and multipotent differentiation capacity (Bi et al., 2007). Other cell types include smooth muscle cells, chondrocytes, vascular endothelial cells, and synovial cells (Kannus, 2000, Sharma and Maffulli, 2006).
The fundamental biological function of tendon is transmitting loading from muscles to bones, and the waviness (also referred as crimp) of the collagenous tissue are widely considered to act as “shock absorbers” during early tendon stretching. Crimping formation of the collagen fibers within the fascicle is a typical phenomenon in tendon (Figure 1a and 1d). Before a strain of 2 %, the disappearance of wavy patterns happens in the collagen fibrils (Gathercole and Keller, 1978). At a strain > 2 %, the stress-strain curve gives rise to a linear region, where the collagen fibrils are entirely un-crimped and reoriented with the stretched triple helix (Silver et al., 2001). When collagen fibers are further loaded, the fibrils start to break (Rigby et al., 1959). Macroscopic failure usually happens when the strain reaches 8-10 %. A current viewpoint is that a tendon extension does not go beyond the 4 % limit during daily movement (Józsa and Kannus, 1997). Human tendons are remarkably diverse in mechanical properties with relevance to locations and ages, as listed in Table 1. In general, tendons (e.g. patellar tendon and Achilles tendon) related to power transmission have tough mechanical properties whilst the tendons (e.g. wrist extensor tendon) which have to assist delicate movements have low mechanical strengths. In addition, the mechanical properties of tendons are expected to be weaker in elderly people than young people, due to the deterioration with aging.

1.3 Human Tendon Healing Mechanism

The conventional viewpoint considers that healing process in a tendon is comprised of three overlapping phases. In the first phase (i.e. inflammation), inflammatory cells transfer into the injured site. In the second phase (i.e. repair), extracellular matrix (ECM) is synthesized by proliferated fibroblasts in the injured site. In the last phase (i.e. remodelling), the newly synthesized collagen fibers orientate along the longitudinal direction of the tendon (Sharma and Maffulli, 2005). A recently proposed tendon-healing model suggested that tendon
regeneration process is attributed to two cell types, which are alpha smooth muscle actin (αSMA) expressing extrinsic cells, and intrinsic tenocytes (Howell et al., 2017). Moreover, distinct mechanisms have been reported to be underlying the neonatal and adult tendon regeneration. Neonatal regenerative tendon healing is initiated by transient recruitment of the extrinsic αSMA-expressing cells, and follows by tenocyte recruitment, differentiation, and restoration of function. On the other hand, adult tenocytes are only activated without recruitment. The absence of tenogenic recruitment might induce aberrant differentiation of tenocytes toward cartilage, leading to permanent scars, poor adult tendon healing, and increased possibility of future re-rupture (Howell et al., 2017). Additionally, the increased concentration of residual collagen type III (COL-III) in the wound site, with thinner diameter and disordered organization, also results in the decline of the mechanical strength (Liu et al., 1995). The entry of foreign cells into the wound site also causes increased types of cells which are not typically present in healthy tendons (Sharma and Maffulli, 2005).

2. Design of Tendon Tissue-Engineered Scaffolds

Owing to the limitations of the biological grafts and artificial prostheses, and the deteriorating imbalance of source and demand of transplants due to population aging, tendon tissue engineering (TE), being an alternative, has boomed in recent decades. TE approaches can be categorized into “top-down” and “bottom-up” methods. In tendon TE, “bottom-up” approaches are more commonly used instead of “top-down”, since single fiber design is usually the first step on the building of increasingly complex scaffolds. Tendon TE has four major advantages including imitation of the hierarchically and anisotropically aligned structure of tendon ECM in various scales ranging from nanometer to micrometer level, reflection of tendon mechanical and nonlinear biomechanical properties, and representation of the essential topographical feature for tenogenic differentiation of stem cell without
biochemical supplementation (Laranjeira et al., 2017). Figure 2 illustrates the typical methodology for tendon TE. The design of repair strategies should closely refer to the characteristics and functions of the natural tendon including anatomical, histological, biochemical and biomechanical properties.

2.1 Biomimicry of Hierarchical Fibrous Structures

Tendon tissues consist of intensively packed aligned collagen fibrils, which in turn organize to higher levels of collagen bundles spatially. These multiple hierarchical levels of collagen fibrous structures assemble from the nano- to macroscale (Kannus, 2000). Most frequently, the loss of tendon functionality is attributed to poor rearrangement of collagen fibrils formed during healing, a typical characteristic of scar tissue, leading to significant weakening of their mechanical properties (Laranjeira et al., 2017). Hence, the imitation of native fibrous organization is crucial, particularly for mechanical properties of tendon scaffolds. One important direction of the field of tendon TE is the development of highly sophisticated three-dimensional (3D) systems aiming at recapitulating the hierarchical and anisotropic fibrous architecture of tendon tissues, which results in the utilizations of different fiber-based techniques.

The selection of techniques and processes is crucial to mimic the complicated fibrous structure of native tendons ranging from nanometer up to millimeter scale. For instance, electrospinning serves as an ideal method for nanofiber generation which replicates the collagen fibrils (Erisken et al., 2012, Yin et al., 2010). Techniques for microfiber fabrication are usually selected to mimic the collagen fibers with the diameter < 100 µm (Wu et al., 2015b, Kew et al., 2012, An et al., 2012). Regarding the replication of fibrous structure with higher level, such as fascicles, multiple fibers could be collected next to each other to form bundles during extrusion/printing process (Kew et al., 2012). It is worth noting that textile
processing is gaining popularity due to its ability to produce hierarchical structures in millimeter level in one or more steps after fiber preparation (Lu et al., 2005, Younesi et al., 2014). In spite of the fact that thicker fiber can exhibit stronger mechanical strength, nano- and microfibers are able to imitate and promote cellular alignment which is comparative to native tendon tissues (Chan et al., 2012). In contrast, inferior cellular reorganization is observed for thicker fibers with a size > 100 μm (Aubin et al., 2010).

2.2 Mechanical Properties

As a crucial component of human musculoskeletal system, the role of tendon is force transmission, which makes the mechanical strength the upmost important properties of tendons. Tendons show a wide range of ultimate tensile stress and Young’s modulus, primarily depending on their locations and functions. In terms of tendon scaffolds, they are required to maintain their shape during implantation, and support the engineered tissue against loads until enough host tissue regenerates (Chan and Leong, 2008). Additionally, flexibility is an important character for scaffold to withstand the mechanical stimuli in vitro/vivo. Generally, high level of porosity usually results in decreased mechanical performance by the introduction of large void space. Therefore, balance between porosity and mechanical strength should be considered (Laschke et al., 2006). Appropriate materials and fabrication technique should be selected accordingly to closely match the range of the mechanical properties of the target tendon type.

2.3 Porosity

The porous structures play a vital role in allowing fluid flow, vascular ingrowth and cells penetration, therefore promote the outcome of TE treatments. A pore size should be larger than 100-250 μm to ensure sufficient vascularization (Laschke et al., 2006) and tissue ingrowth (Lu et al., 2005). A porosity of 60-70 % and a good interconnectivity are also
needed in TE applications (Hollister, 2005). Particularly, fibers are densely assembled in most of the cases for tendon TE, and cell infiltration and its correlation with porosity of the scaffold are only considered in a few studies (Laranjeira et al., 2017, Wu et al., 2016a).

2.4 Collagen Fiber Crimp

A tri-phasic mechanical behaviour is a typical characteristic in the stress-strain curve of tendons, which is comprised of toe, linear, and yield regions. The basis of this pattern is relevant to crimp structure at the fascicle level of tendon. Research suggested that the actin stress fibers and the cell nuclei organize following the course of collagen crimps within the native tendon, which actively regulate alignment of collagen fibers (Ralphs et al., 2002). Aligned contractile stress fibers are able to recover cells from stretch during mechanical loading, and modulate mechanotransduction signals with the help of gap junction network within tenocytes (Ralphs et al., 2002). Considering the importance of crimped fibers to the mechanical properties and biological function of tendons, inclusion of such structures should be taken into account in the design of tendon scaffolds. The biomimicry of crimp structure or its induced mechanical outcome is usually achieved by crimped fiber fabrication or tuning the angles of braided fiber bundles (Wu et al., 2017e, Czaplewski et al., 2014, Surrao et al., 2012).

2.5 Biomaterials for Tendon Scaffolds

In general, scaffolds can be built by synthetic materials and/or natural materials. In tendon TE, aliphatic polyesters including polylactic acid (PLA), polyglycolic acid (PGA), their copolymer (i.e. poly(lactide-co-glycolide), PLGA) and polycaprolactone (PCL) are the most popular polymers due to their superior mechanical strength. PGA is mechanically stronger than PLA, and the mechanical features of PLGA could be tailored by altering the ratio of glycolic acid (GA) and lactic acid (LA) (Lu et al., 2005). Scaffolds fabricated using PCL,
PLA and PGA show that PCL is tougher than PLA (Czapelewski et al., 2014), and weaker than PGA mechanically (Aghdam et al., 2012). In terms of degradation, PGA scaffolds have been reported to have a rapid mechanical strength decline 2 to 4 weeks after implantation (Ratner et al., 2004), which is insufficient for tendon repair. As compared to PGA, PLA has slower degradation rate (Lu et al., 2005), but the hydrophobility limits its application (Ratner et al., 2004). PCL degrades at a slower rate compared to PGA and PLA, which manifests a complete degradation over a period of 2-4 years (Woodruff and Hutmacher, 2010), and is able to main its mechanical properties in vivo for more than 6 months (Lam et al., 2009).

With regards to natural materials, collagen is the most ideal choice for tendon TE owning to its constituent similarity to native tendon. However, collagen is weak in its initial mechanical properties, which is also affected by the source, preparation/purification conditions and process steps (Kew et al., 2012). The crosslinking chemistries for collagen such as 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and epoxy compound ethylene-glycol-diglycidyl-ether (EGDE), have been extensively investigated to improve the properties of the extruded collagen fibers (Zeugolis et al., 2009, Enea et al., 2011). Alginate, as an alternative of collagen, is a naturally existent anionic polymer, and has also been applied for tendon TE (Majima et al., 2005), due to its biocompatibility, low cost, and convenient crosslinking by exposure to Ca^{2+} ions (Lee and Mooney, 2012). Moreover, silk from silkworms has been the most popular natural material for tendon TE so far due to its structural similarity and superior mechanical strength. Silk exists in the form of fibers which is very flexible for textile processing without the need of fiber formation. In addition, silk possesses Young’s modulus in the scale of GPa and ultimate tensile stress of hundreds of MPa, which are several orders higher than other natural biomaterials (Vepari and Kaplan, 2007). The major constrain of silk in tendon TE application is its slow degradation rate and mismatch between weight loss and decline of mechanical properties (Wu et al., 2017d, Fang et al., 2009).
chitosan and hyaluronan are also used to adjust the biological functions of the tendon scaffolds (Funakoshi et al., 2005a, Laranjeira et al., 2017).

In order to make up for the limitations of a single material, researchers have combined different synthetic materials, natural materials, or natural and synthetic polymers. Scaffolds fabricated by material blends integrate the advantages of each complementary material, and exhibit up-regulated biocompatibility and mechanical properties. Some examples of such combinations for tendon TE include PCL-PLA (Rothrauff et al., 2017, Czaplewski et al., 2014), collagen-silk (Chen et al., 2008, Zheng et al., 2017), PCL-chitosan (Zhao et al., 2015, Domingues et al., 2016), PCL-gelatin (Yang et al., 2016), and PLGA-silk (Sahoo et al., 2006).

2.6 Cell Source for Tendon TE

The cell source is a critical consideration since improper selection of a cell source can also result in failed tenogenesis/tendon regeneration, phenotypic drift toward osteogenesis leading to ectopic bone formation. Various cell types have been explored for tendon TE, with tenoblasts and tenocytes being most widely used. However, harvesting autologous tenocytes is challenging due to their limited cell number and the risk of donor site morbidity (Yang et al., 2013). Since dermal fibroblasts are relatively abundant in human body (Sriram et al., 2015) and have multilineage differentiation potential (Lorenz et al., 2008), the use of dermal fibroblasts has also been explored for tendon repair (An et al., 2012, Gigante et al., 2009). Moreover, dermal fibroblasts share common characteristics with tenocytes, as both cell types are mature and originated from mesoderm (Rodrigues et al., 2013). Alternatively, stem cells are very popular in TE applications due to their multipotency. Mesenchymal stem cells (MSCs) have been widely utilized for numbers of tendon TE applications. However, how to avoid ossification when differentiating MSC into tenogenic lineage remains a great
challenge (Yin et al., 2010). Also, MSCs become scarce with increased age (Diekman et al., 2012). In addition, human embryonic stem cells (hESCs) (Chen et al., 2010) and induced pluripotent stem cells (iPSCs) (Czaplewski et al., 2014) have also been considered for tendon TE, which were differentiated to MSCs prior to cell seeding. In some studies, human tendon stem/progenitor cells (hTSPCs) and adipose stem cells (ASCs) were also reported to be cell sources for tendon TE (Yin et al., 2010, Yin et al., 2013, Laranjeira et al., 2017).

2.7 Cell Alignment on Tendon Scaffolds

In highly anisotropic tendon tissue, cells align along the direction of collagen fibers with the elongated nucleus occupying almost entire length of the cell (Kannus, 2000). Actin cytoskeleton is reported to be a key element in connective tissue development. The alignment of cytoskeleton triggers nucleus deformation, leading to the alteration of gene expression (McNamara et al., 2010). The high cellular alignment and elongation triggered by aligned topography of the scaffolds, have been proven to direct the tenogenic differentiation of stem cells (Yang et al., 2016, Chen et al., 2010, Rothrauff et al., 2017). Topographical cues such as ridges (Kim et al., 2009), pillars (Li et al., 2013), channels (Lee et al., 2006) and fibers (Kew et al., 2012) are known to lay crucial foundation for the alignment and elongation of cytoskeleton in nanometer and micrometer scale. More importantly, the effect of growth factors on tenogenic differentiation is only observed at the gene expression level rather than on matrix production (Tan et al., 2012, Chai et al., 2013, Younesi et al., 2014), indicating growth factor has a limited role in tenogenic expression, which further emphasizes the importance of topographical cues. Considerations for scaffold design in tendon TE are summarized in Table 2.

3. Current Fiber-Based Techniques for Tendon TE
Various technologies which allow fabrication of 3D scaffolds have been developed owing to the progresses in engineering, material science, chemistry and biology. The 3D scaffolds are able to closely replicate the anatomical features and mechanical characteristics of the native tendon, and simultaneously, enable localised and sustained delivery of drugs (Kew et al., 2011). Technologies for scaffold fabrication include freeze-drying (Ho et al., 2004), gas foaming (Chung et al., 2011), solvent casting and particulate leaching (Katoh et al., 2004), rapid prototyping (Sachlos and Czernuszka, 2003), and fiber-based techniques (Heinemann et al., 2009). For tendon TE applications, fiber-based techniques are the most popular scaffold fabrication approaches since they facilitate the imitation of fibrous organization of the tendon.

General applications of fiber-based techniques in TE have been reviewed elsewhere (Tamayol et al., 2013). The objective of this review is to focus on the state-of-the-art strategies in the field of tendon TE. The anatomical characteristics of native tendon (structure, composition, architecture and topography) have been introduced in the first section, inducing the discussion of requirements for design of tendon TE scaffolds. In terms of the conventional techniques and processes such as electrospinning, knitting and braiding, new scaffold designs in recent years have also been included in this review. In addition, more techniques, which were reported recently and have showed promising results for tendon regeneration, have been involved in this review, including electrochemical alignment technique, electrohydrodynamic jet printing and microfiber melt drawing, and the resultant physical and biological outcomes have been summarized and compared with the native tendon tissue. More specifically for tendon TE, techniques for crimped fiber fabrication are also discussed.

3.1 Electrospinning
Electrospinning is the most popular technology to fabricate fiber-based scaffolds for TE applications. In electrospinning process, a flow of polymeric material ejects out of a nozzle, and is subjected to an electric force between a nozzle and a collector to form fibers (Figure 3a) (Deng et al., 2012). Random and oriented fibers with the size ranging from hundreds of nanometers to several micrometers can be collected using a stationary substrate and particularly designed setup (e.g. rotating mandrel, parallel electrodes and disc collector) (Teo and Ramakrishna, 2006), respectively.

In one study, Moffat et al. (2008) reported PLGA scaffolds using electrospinning. Scaffolds with unaligned fibers (~620 nm in diameter) collected by a stationary plate and aligned fibers (~570 nm in diameter) by a rotating mandrel were fabricated. The rotator cuff fibroblasts grown on the aligned fibers were oriented whilst cells seeded on the unaligned fibers showed an isotropic morphology. Up-regulated expressions of α2 integrin and COL-I were detected in aligned scaffolds. In another study, Xie et al. (2010) created an electrospun PLGA scaffold that contained both random and aligned fibers using the separated-plate setup. Rat tendon fibroblasts proliferated well, and collagen expressed on both aligned and unaligned fibers. Similar to results in Moffat’s study, the cells seeded on aligned fibers presented an extended spindle shape parallel to the course of the fibers, while cells seeded on random fibers displayed a disordered actin cytoskeleton. In addition, in both studies, the mechanical testing results showed the scaffolds with aligned fibers had tougher mechanical strength than those with random fibers.

Effort of enlarging the pore size of electropun fibers have been reported by incorporating the aligned and random fibers together. Yang et al. (2014) used silk fibroin and poly(l-lactide-co-caprolactone) (P(LLA-CL)) composites to develop an electrospun scaffold using a two-collector system, which was a rotating mandrel supported on a linear guide slider. The
scaffold which comprised yarns (~30 µm) with aligned nanofibers (~900 nm) in tandem with randomly distributed nanofibers connecting the yarns, exhibited a 3D network with fairly large pores (~550 µm²) compared to the scaffolds with only either random or aligned fibers. Culturing of the bone marrow-derived MSCs exhibited that the scaffold could promote cell proliferation rate and infiltration. The tensile testing results also indicated that the aligned yarns reinforced the mechanical strength of the scaffold.

In a recent study, Domingues et al. (2016) incorporated cellulose nanocrystals (CNCs) into electrospun nanofiber scaffolds using distinct ratios of CNCs (1, 3 and 6%) based on a polymer blend of PCL and chitosan. The biomaterial-toughing effect was increased by 85% and the scaffolds mechanical properties was enhanced to match the native tendon properties with modulus of 540 MPa and tensile stress of 40 MPa. Unlike randomly oriented scaffolds, the topography of anisotropically aligned scaffolds achieved a significant uniaxial cell orientation and elongation with human tendon cells in vitro.

In order to modulate the chemical, physical and biological performance of the scaffolds, co-electrospinning was applied. Multiple nozzles from different directions were used concurrently to deposit more than one materials onto the collector. As a composite scaffold, PCL fibers of 1.2 µm and chitosan fibers of 360 nm were co-electrospun for treatment of rotator cuff tears by Zhao et al. (2015). Interestingly, the composite scaffolds represented remarkably enhanced strength and failure strain than chitosan scaffolds as well as improved stiffness than PCL scaffolds. The enhanced wettability of PCL-chitosan scaffolds gave rise to increased fibroblast attachment and proliferation than PCL scaffolds alone, leading to improved neo-mineralization, collagen and GAG expression. These features were confirmed in vivo with the tissues at the tendon-bone insertion site using PCL-chitosan scaffolds in the rats study.
In another study, Yang et al. (2016) generated a composite scaffold with co-electrospinning of methacrylated gelatin (GelMA) and PCL. By dissolving and photocrosslinking the electropsun GelMA fibers throughout the scaffolds, a uniform distribution of GelMA was preserved without declined mechanical strength of the scaffold. Consequently, multilayered constructs were formed with stacked scaffold sheets being connected by photocrosslinking. Interestingly, under the regulation of transforming growth factor (TGF-β3) and topographical cues, impregnated human ASCs in the structure gained orientation, leading to the upregulated gene expression of Scleraxis and tenasin-C. With a similar concept, Wu et al. (2017a) developed an hybrid electrospinning and electrospraying process to produce functionally graded scaffolds. The dispensing conditions of PCL and hydroxyapatite were adjusted layer by layer. The morphology, C/Ca ratio, layer thickness, mechanical properties of scaffolds, roughness and water contact angles in different layers were observed to vary gradually with the change of the dispensing conditions of the materials, indicating this simple and versatile method is able to directly fabricate scaffolds with functional gradient for tendon-bone interface regeneration.

Electrospinning is of great advantage to scaffold fabrication, since it is relatively simple to set up, efficient to control the key process parameters, applicable for a large variety of biomaterials, possible to scale-up (Deng et al., 2012) and capable to build scaffolds with high porosity. The process, however, is difficult to use in the manufacturing of a 3D structure with certain thickness. Furthermore, the high density of fiber packing results in small pore sizes and limited cell migration into the constructs (Leong et al., 2010).

3.2 Electrohydrodynamic Jet Printing (E-Jetting) Technology

Electrohydrodynamics (EHD) refers to the study of the dynamics of electrically charged fluids (Castellanos, 1998), which is the theoretical basis of the EHD printing technologies.
With the assistance of the an electric field, a hemispherical droplet at the nozzle tip transforms to a conical shape, termed “Taylor Cone”, and then forms a liquid jet once the electric field force surpasses the surface tension of the droplet (Saville, 1997). EHD printing can be classified to be EHD jet patterning, electrospinning and electrospraying according to the jet mode (Jaworek and Krupa, 1999). The most commonly used EHD printing process for TE applications is electrospinning, which has been introduced in Section 3.1. A technology termed near field electrospinning (NFES) or e-jetting is rooted in traditional electrospinning, and rapidly developed in the recent decade (Huang et al., 2013). The reduced nozzle-to-substrate distance in electrospinning prevents the fiber from bending instability before it deposits on the collector, which makes it possible to manipulate a single fiber (Figure 3c).

Our group has developed a 3D porous scaffold for tendon TE application, comprising two portions (i.e. inner and outer portions) (Wu et al., 2016a, Wu et al., 2017c, Wu et al., 2015a). The outer portion rolled from an e-jetted PCL fiber mesh, and comprised a tubular multilayered structure with aligned microfibers (~25 µm) and large pores (~110 × 2000 µm) (Figure 3d). The inner portion was fabricated by uniaxially stretching a heat-pressed PCL tube, and displayed an orientated micro-ridges/groove array (ridge length: ~120 µm, inter-ridge distance: ~5 µm) along the scaffold longitudinally. The mechanical strength of the scaffold (Young’s modulus: ~227 MPa, ultimate tensile stress: ~50 MPa) was similar to the human patellar tendon. Culturing of human tenocytes showed a significantly increased cellular proliferation in the scaffolds when compared to electrospun scaffolds. Furthermore, both outer and inner sections of the scaffold facilitated the cells to align and elongate with controlled orientation, as well as up-regulated expression level of tendon-related proteins (e.g. COL-I, decorin, biglycan and tenascin-C). In addition, a hydrolysis study showed that the scaffold degradation exhibited consistency between the weight loss and the decline of
mechanical properties, indicated by a 65% decrease in ultimate tensile strength, with a corresponding 60% loss in mass after 30 days.

3.3 Electrochemical Alignment Technique

In 2008, Akkus’s group presented the electrochemical alignment technique to assemble COL-I molecules (Cheng et al., 2008). The key feature of this technique is to insert linear electrodes into the collagen solution so that electrolysis of water molecules give rise to a pH gradient between electrodes. The collagen molecules behave in an ampholytic way, in which the net charge near the cathode turns negative, and that near the anode turns positive. When the external electric field is applied, charged collagen molecules will keep migrating until they reach the isoelectric point, resulting in a congregation within a plane (Figure 3e) (Uquillas and Akkus, 2012). The obtained aligned collagen bundles were then treated with phosphate buffer saline (PBS) to promote fibrillogenesis, followed by crosslinking in genipin. In this pioneer study, they were able to create collagen bundles with the size of 50-400 mm (Figure 3f), with mechanical strength 30-times stronger than randomly oriented-crosslinked collagen gel, which was comparative to the native tendon. The twisted collagen bundles were also conductive to proliferation, orientation and migration in inter-bundle spaces of the tendon-derived fibroblast cells.

More recently, their research group developed electrochemically compact collagen sheets with planar electrodes, and investigated the effect of stiffness anisotropy (SA) of the collagen sheets on stem cell fate by altering SA from 1 to 8 using a stretching device (Islam et al., 2016). The collagen sheets with different SA displayed distinct mechanical properties and morphology in transverse and longitudinal directions. Higher cytoskeletal aspect ratio of seeded MSCs was observed as the SA increased. Cells also showed upregulated expression levels of tendon-related markers including Scleraxis and Mohawk in the SA-dependent
fashion. After a culture period of 21 days, upregulated levels of other tendon-related markers such as thrombospondin-4 and COL-I, and III were also induced, indicating the significant effect of SA on MSC differentiation.

The advantages of the electrochemical process include no utilization of toxic solvents for collagen fiber preparation, economic efficiency, practicality for batch processing using electrode arrays, and ability to fabricate long rope-like structure (Cheng et al., 2008).

3.4 Other Fiber-Assembly Technologies

Apart from above-mentioned technologies, other fiber-assembly technologies have also been proposed for tendon TE. Considering that collagen is the major composite in native tendon, extrusion of collagen fibers has drawn great interests. Micrometer-size collagen fibers were first manufactured using a wet extrusion system in late 1970s. Since then, a number of studies have showed that this process was able to generate collagen fibers, whose ultrastructure, physical and mechanical features were comparative to the nature tendon (Kato et al., 1989, Pins and Silver, 1995, Zeugolis et al., 2008). Recently, Kew et al. (2012) developed an automated system to assemble collagen/poly(ethylene glycol) (PEG) fibers. The collagen gel was extruded into a bath comprising PBS and PEG to induce coagulation and partial fibrillogenesis, followed by winding the fibers onto a spool (Figure 3g). To fabricate collagen fascicle structure, collagen/PEG fibers were intertwined into a multiple-fiber over-layer structure to generate bundles (Figure 3h). The resultant fiber bundles showed controlled numbers of fibers each fascicle to imitate the microscopic architecture of native tendons.

However, the mechanical properties of extruded collagen fibers were usually too weak for tendon applications. To address this issue, Panas-Perez et al. (2013) have proposed silk-collagen fibrous scaffolds with silk volumes lager than 14 % and collagen volume less than 86 % for ACL reconstruction. The composite scaffolds exhibited greater initial ultimate...
tensile stress as compared to the human ACL. Moreover, the mechanical degradation findings suggested that composite scaffolds were able to partially meet the mechanical requirements necessary for a functional ACL reconstruction.

Besides collagen, chitosan-coated alginate fibers with better mechanical properties than alginate fibers, have been applied in tendon repair via a modified wet spinning method (Tamura et al., 2002). In this method, sodium alginate solution was extruded through the stainless-steel spinnerets (0.1 mm diameter × 50 holes) into the first coagulation bath with CaCl₂ and chitosan, and further crosslinked by second bath with CaCl₂. Coagulated filament was then wound by stretching procedure (Figures 3i and 3j). The in vitro study demonstrated adherence of rabbit tendon fibroblast and COL-I expression in the composite fibers (Majima et al., 2005). Also, chitosan-based hyaluronan composite fibers were fabricated by modifying this wet spinning method (Funakoshi et al., 2005b), in which chitosan was extruded, followed by a three-step bath treatment (i.e. CaCl₂ in methanol, methanol, and hyaluronan in methanol). The prepared fibers were then perpendicularly stacked in six layers to create a scaffold. The introduction of hyaluronan coating resulted in increased mechanical properties, cell adhesion and COL-I expression than the chitosan fibers (Funakoshi et al., 2005b).

Another technique named microfiber melt drawing has been proposed (An et al., 2012). Polymer was heated, manually pulled from the bottom of the holder by a needle, and the fibers were continuously wound onto a rotating collector (Figures 3k and 3l). Aligned PCL fibers with the diameter of 10 μm were prepared without using organic solvent. In vitro study was supportive for microfibers to facilitate the proliferation of human dermal fibroblasts. The fibers were implanted into a rabbit model for Achilles tendon repair. Tendon tissue was observed to infiltrate into the microfibers. Moreover, the restored tendon had a well-defined structure due to the regulation of aligned microfibers.
Differently from abovementioned approaches, a concept of composite living fibers (CLFs) in which a core of load bearing polymeric threads was coated by a hydrogel layer containing cells, has been reported (Akbari et al., 2014). Threads were pulled through a sequence of baths containing CaCl₂ and alginate mixed with cells using a spool, and the thickness of coating layer could be adjusted (Figure 3m). This method has been further explored by Costa-Almeida et al. (2017) for tendon TE. The hydrogel layer of alginate and GelMA that loaded with human tendon cells, was coated on braided surgical suturing threads (Figure 3n). The fibers were able to be assembled using textile processes, in order to enhance mechanical properties. Also, cells were observed to migrate in the hydrogel, align on the threads, and express tendon-related genes and genes involved in matrix remodelling. Table 3 summarizes the studies on these fiber-assembly technologies for tendon TE, and properties and biological outcomes of the fabricated scaffolds.

4. Textile Processing for Complex Fibrous Structures

Textile processing can be applied after the fabrication of the fiber-based material to produce more complex structures and reinforce mechanical strength of scaffolds, which are very attractive to tendon TE. Amongst textile techniques, knitting and braiding are the most popular, and usually used to postprocess scaffolds fabricated by techniques mentioned in Section 3.

4.1 Knitting

In knitting, yarns or threads are intertwined in a series of linked loops to from a fabric. Knitted textile substrates offer a great mechanical properties due to their interlocked structure (Pandita et al., 2002). Owing to the superior mechanical properties and ease of production, knitted scaffolds have been reported in some TE applications such as endovascular prosthetic
device (Freitas et al., 2010), cartilage (Dai et al., 2010, Kawazoe et al., 2010), skin (Ananta et al., 2008, Wang et al., 2012) and blood vessels (Ravi and Chaikof, 2010).

Ouyang et al. (2003) have developed a knitted PLGA scaffold seeded with bone marrow stromal cells (BMSC) for Achilles tendons regeneration. Animal work in the New Zealand White Rabbits (NZWR) indicated that the wound sites healed well, and COL-I and III fibers were observed in the regenerated tissue. In addition, the tensile strength and modulus of healed tendons reached ~85% and ~60% of the native tendon, respectively. Such approach was further improved by Chen et al. (2008), who incorporated knitted silk scaffold with the collagen matrix, and investigated in rabbit medial collateral ligament (MCL) defect model. Wound sites cured with a silk-collagen scaffold showed increased collagen matrix deposition, superior mechanical properties and larger diameter collagen fibrils than the untreated wound sites and those treated with silk scaffolds. In another similar study, Chen et al. (2010) incorporated MSCs derived from hESC within a knitted silk-collagen scaffold (Figure 4a). When the scaffolds were exposed to mechanical stimuli in vitro, hESC-MSCs presented similar morphology as tenocytes, and expression of tendon-related gene markers including Epha4, COL-I, COL-III and Scleraxis.

In the most recent study, Zheng et al. (2017) fabricated a 3D aligned collagen/silk scaffold (ACS). The collagen was oriented using a unidirectional freezing technique, in which the collagen was frozen from one end to another gradually, and surrounded the knitted scaffold. ACS displayed similar aligned arrangement of collagen fibers as native tendons. TSPCs showed spindle-shaped and well-aligned morphology on ACS, and were longer than those on sponge collagen/silk scaffold in vitro. In an in vivo rabbit rotator cuff repair model, denser regenerative connective tissues and abundant collagen fiber bundles with more organized arrangement were formed in the ACS as compared to the tiny and disorganized collagen
fibers inside the sponge collagen/silk scaffold. Also, more cells were found inside the ACS, indicating cell infiltration. ACS group also displayed higher tenogenic differentiation (e.g. COL-I, III, tenascin and biglycan) after 4 weeks’ surgery, and greater diameter collagen fibrils (~50 and ~45 nm, respectively) at 12 weeks post-implantation. In addition, biomechanical testing results revealed that ASC was mechanically tougher than sponge collagen/silk scaffold in terms of failure load (~140 and ~100 N), ultimate stress (~4.4 and 3.4 MPa) and energy (0.42 and 0.26 J) at 12 weeks after implantation.

Apart from the adequate mechanical properties and ease of building 3D geometries, knitted scaffolds are able to adjust its physical and mechanical properties by varying the pore size, which makes it attractive to construct porous tissues. However, knitted scaffold was reported to require gel systems for cell seeding (e.g. fibrin or collagen gel), which declined the pore size (Sahoo et al., 2006). Also, it was observed that tendon restoration in knee using knitted scaffold with gel was unsatisfactory due to the dissociation of the cell-gel composite during movement (Ge et al., 2005).

4.2 Braiding

In braiding, three or more fiber strands are intertwined to produce complex structures in the shape of cylinders and rods, which are suitable for engineering tendon scaffolds. Cooper et al. (2005) reported braided scaffolds manufactured from PLGA fibers, which consisted of the attachment sites at the two ends of the body region (Figure 4b). PLGA fibers were laced to create yarns with certain density, followed by being placed in a braiding machine. The braiding machine used the sequential motion of the carriers to generate 48-yarn, with braiding angles ranging from 26° to 31°. Some design parameters including the fibrous structure, porosity, degradability and cell types, were studied to obtain an optimum braided scaffold.
In another study, Lu et al. (2005) braided three types of aliphatic polyester fibers, namely PGA, PLA and PLGA to mimic the ACL. It was observed that cell morphology was dependent on the materials. According to an in vitro degradation study, PGA scaffold lost its integrity after 2 weeks, which was significantly faster compared to PLGA and PLA, and too fast to be used for tendon repair. The rapid degradation of PGA led to matrix disintegration and cell death in a short period. In contrast, some small cracks and debris were observed on the PLGA samples in the same duration. The surface of PLA fibers remained fairly intact, and the scaffolds could maintain their structural stability and mechanical properties in prolonged culturing period.

Fang et al. (2009) braided scaffolds using pernyi silk fibroin, seeded the scaffolds with tenocytes and then implanted them into adult NZWR. The implanted scaffold was observed to integrate with the neighbouring tissues successfully. The maximum load of neo-tendon after 16 weeks of in vivo implantation reached 55% of the native tendon. However, only the external section of the silk fibroin displayed degradation at 16 weeks. More recently, Walters et al. (2012) reported COL-I scaffolds for ACL replacement by braiding the crosslinked collagen fibers. The tensile testing results showed remarkably reduced mechanical properties of the scaffolds including ultimate tensile strength, viscoelastic properties and Young’s modulus due to the addition of gelatin.

In one more recent study, Czaplewski et al. (2014) developed braided submicron fibrous scaffolds using PLA, PCL and their blends. Various fiber chemistry (i.e. blend ratios) and braiding angle (i.e. number of stiches/inch) were observed to determine the mechanical properties. Unsurprisingly, PLA was more superior for adhesion and ingrowth of hiPSCs-derived MSCs than PCL. Remarkably, with the exposure to aligned topography, cyclic mechanical stimuli and no aid of differentiation factors, the scaffolds braided with large
angles displayed upregulated expression of tendon relevant markers, fibroblast-like, spindle cell morphology in company with decline of osteogenic markers, indicating superior ability in facilitating hiPSC-MSC tenogenic differentiation than those with small angles.

In addition to the capability of mimicking fiber arrangement and tolerance to different types of mechanical loads, the porosity and the mechanical properties of the braided scaffolds are also adjustable spatially (Barber et al., 2011). However, the porosity of the braided scaffolds is lower than their knitted counterpart (Tamayol et al., 2013), and pore size of the braided scaffolds are small in fiber strands due to the densely packed fibers.

4.3 Combination of Processes

Since the aforementioned technologies were limited by their respective disadvantages, researchers usually combined some of them together to promote the performance of the scaffold. For example, Sahoo et al. (2006) created a scaffold by electrospinning PLGA nanofibers on the surface of a knitted PLGA scaffold leading to an enhanced surface area for cell attachment, and slightly improved mechanical strengths. Porcine BMSCs were seeded to investigate the cell response compared with the fibrin gel-based knitted scaffold. The hybrid scaffolds showed enhanced cell proliferation and increased expression of several tendon-related markers (i.e. COL-I, decorin and biglycan). In another study, Sahoo et al. (2010) fabricated a bio-hybrid scaffold by electrospinning PLGA fibers released with the basic fibroblast growth factor (bFGF) over a knitted silk mesh. Upon seeding with mesenchymal progenitor cell (MPC), the results showed that bio-hybrid scaffold not only promoted cell proliferation and attachment on both electrospun PLGA fibers and silk fibers, but also triggered collagen production and tendon-specific gene expression.

Braiding technique has also been incorporated with electrospinning. Barber et al. (2011) developed scaffolds by braiding the aligned electrospun PLA fibers with 3, 4 or 5 bundles.
The stress-strain curve indicated the toe region, which presents in native tendons. The results of mechanical testing varied in scaffold with different numbers of bundles, suggesting the mechanical properties can be modified by the change of the braided structures. The result of seeding hMSCs on the scaffolds showed the cells were able to align along the length of nanofibers.

In a latest study, aligned nanofibrous sheets of PCL or PLA fabricated from electrospinning were assembled to form multilayered braided or stacked scaffolds for tendon TE (Rothrauff et al., 2017). Braided scaffolds had stronger tensile and suture-retention strengths at a cost of decreased moduli. Upon seeding the human bone marrow-derived MSCs, both scaffold designs supported tenogenic expression, with greater effect on braided scaffolds. In contrast, stacked structure exhibited advantage in cell infiltration, which led to increased cell number, total sulfated GAG and collagen content, but the relative depositions were comparable when normalized against dsDNA content. In addition, both scaffolds supported expression of tenogenic markers, while those in braided scaffolds showed more up-regulation as compared to the stacked ones.

Furthermore, Laranjeira et al. (2017) successfully imitated the collagenous ECM of tendon by braiding and interweaving aligned electrospun nanofiber threads based on electrospinning PCL/chitosan/CNC nanoscale fibers into 3D hierarchical scaffolds (Figure 4c). The braided scaffolds showed a higher Young’s modulus and ultimate tensile stress than the woven structures, and at the same time, the woven scaffolds had higher toughness and tensile load. The stress-strain curve of the woven structures exhibited nonlinear toe region as native tendons at low strains (< 6%). In the in vitro test on the woven scaffolds, in addition to cytoskeleton elongation and anisotropic organization, tendon-related markers including
Tenascin-C, COL-I, III and Scleraxis were found to express on the scaffold upon seeding ASCs and human tendon derived cells.

Wet-spun fibers have also been braided in a study of Funakoshi et al. (2005a). The threads of chitosan-based hyaluronan fibers were twisted and braided with a braiding angle of 100° to form the scaffolds. In a rabbit study in vivo, postoperative results of 4 and 12 weeks revealed an increased amount of well-organized COL-I fibers in company with improved tensile strength and modulus in the fibroblast-seeded scaffolds.

Interestingly, knitting and braiding techniques have also been integrated to fabricate tendon scaffolds. Fan et al. (2009) fabricated an ACL scaffold by rolling a knitted silk mesh with silk sponge coating around a braided silk core (Figure 4d), which aimed to obtain sufficient mechanical properties in large animal model. They seeded the scaffold with MSCs and implanted the cell-laden scaffolds into pig model. The regenerated neo-tissue exhibited fibroblast-like morphology, and expressed genes of tenascin-C, COL-I and III. Also, the biomechanical testing results indicated that the scaffold was capable of enduring 51.8 % of maximum load of the native pig ACL, which was comparable to the value in normal physical activity.

On the basis of the concept of electrochemical alignment, Younesi et al. (2014) customized a rotating electrode device to assemble collagen threads in continuous length that replicated the structure and mechanical features of native tendons. The collagen threads were further pin-woven and sutured to tendon scaffolds, which have a similar load-displacement behavior and mechanical properties to native tendons (Figure 4e). Furthermore, its relatively high porosity of 80% enabled cell seeding within the continuum of the biotextile. Interestingly, unlike randomly oriented collagen gel, the seeded MSCs experienced tenogenic differentiation and synthesized a matrix expressing COMP, tenomodulin and COL-I in the absence of growth.
factors. Table 4 summarizes the studies which used textile techniques for tendon TE, and properties and biological outcomes of the fabricated scaffolds.

5. Scaffolding Techniques for Crimped Fibers

Crimps of the collagenous network are a typical feature in the tendon matrix, permitting 1-4 % elongation of individual fiber without tissue injury (Józsa and Kannus, 1997, O'Brien, 1997). Although it is well-known that the crimp structure is critical in tendon, only a few studies have been reported to fabricate the crimp-like fibers. The modified electrospinning process is able to print coil-like patterns (Xin and Reneker, 2012, Hellmann et al., 2009, Han et al., 2007), when a translational movement at a certain range of speed is introduced into the electrospinning process. However, the process suffers from a poor repeatability, which is caused by the uncertainty of the swing direction and the frequency of the jet path (Hellmann et al., 2009). Also, when this process is scaled up to multiple layers, the pores are overlapped due to the uncontrollable fiber orientation.

In one study, researchers have electrospun the poly(L-lactide-co-ε-caprolactone) (PLDLLA), and collected fibers using a rotating wire mandrel setup, which was assembled by two circular pieces connected with four equally spaced rods (Surrao et al., 2012). Crimp-like fibrous scaffolds were attained by immersing the electrospun scaffolds in PBS solution with a temperature of 10 °C higher than the glass transition temperature of the polymer. When the residual stress in the fibers, which was caused by the stretching during electrospinning process, was released, the fibers shrank in length and formed wavy microarchitectures. The induced wave pattern was similar to that of the collagen crimp with amplitude of 5 µm and wavelength of 46 µm.

In another study, crimped collagen fibers were manufactured using micro-patterned elastomeric substrates as a template (Caves et al., 2010). A flexible polyurethane micro-
ridge mould was fabricated using the photolithographic technique. Collagen fibers prepared by fiber spinning were sandwiched between a flat substrate and the micro-ridged mould, which pre-extended to a strain of 15 to 55%. The extended mould was released to induce collagen fibers with micro-crimped features, followed by collagen crosslinking with glutaraldehyde vapor (Figures 5a-5d). Sequentially, fibers were embedded within an elastin protein matrix, and sheets with fiber reinforcement were achieved by a thermal sol-gel process (Figures 5e and 5f). The collagen fibers revealed a repetitive crimped pattern with the degree of crimp (e.g. ~3-9%, Figure 5g), which was directly correlated to the extent of pre-extension (15-30%).

Recently, crimped fibers have also been printed by e-jetting (Wu et al., 2017e, Wu et al., 2017b). Through optimization of the process parameter (i.e. segment length, stage speed and dwell time), the printed fibers exhibited controllable and regular morphologies with a crimp angle of ~15° and fiber diameter of ~45 µm (Figures 6a and 6b), which are comparable to those in the native tendons. The stress-strain curve of the crimped fibers displayed an initial nonlinear region and a subsequent linear region with different Young’s modulus (22.6 ± 2.2 MPa and 33.2 ± 6.5 MPa, respectively). The cellular alignment analysis demonstrated that cell orientation could be regulated by the crimped fibers with a broader and controllable nuclei angle distribution, as compared to those on the straight fibers.

6. Summary and Future Perspectives

Various approaches have been applied in scaffolding for tendon TE, and their performances in generating functional, anatomically similar, physiologically relevant, mechanically and structurally stable, and biologically appealing constructs vary considerably. Fiber-based techniques offer many inherent benefits in tendon tissue repair. First, they are capable of providing microstructures to mimic the native collagenous matrix and regulate
cell response. Secondly, there are a wide variety of synthetic and natural polymers, which are processible using fiber-based methods, and are able to produce anisotropic geometry, proper mechanical properties, porosity, fiber diameter, and controllable degradation rate. The studies reviewed here demonstrate the promise in the field of fiber-based scaffolds for tendon TE. Having reviewed in detail the various processes of engineered tendons and their advantages and disadvantages, it is critical to look at the realistic utilization potential of these methods clinically. No technology is perfect and all of them suffer from certain limitations and challenges.

Although a lot of advance in fiber-based tendon TE has been achieved, some scaffold fabrication issues are still waiting to be overcome for the widespread clinical utilization. For example, tissue formation could be guided and/or enhanced by the delivery of bioactive reagents (e.g. growth factors) in tandem with the scaffolds. However, polymer dissolution in toxic solvents or exposure to high temperature is required in current fiber-based processes (e.g. electrospinning, wet-spinning and e-jetting) (Brown et al., 2011), which hinders the integration of either cells or biomolecules. Therefore, integration of bioactive elements into fiber-based techniques for tendon TE applications, keeping them biologically stable and active, and simultaneously regulating their functionalization still remain challenging.

One solution to address this issue could be utilizing the 3D bioprinting techniques to deliver the cells and other bioactive materials within hydrogel systems. 3D printing approaches have advanced considerably in the past decade, in which cell-laden hydrogel is printed with or without supporting structures. For example, Yeo and Kim (2014) developed a hybrid scaffold comprising extruded micrometer-sized PCL fibers and electrospun PCL nanofibers in tandem with cell-laden alginate struts. In another study, Wu et al. (2016b) reported a study about the
3D printing of human corneal epithelial cells (HCECs) within a collagen/gelatin/alginate composite hydrogel, and obtained cell-laden tissue structure with controllable degradation.

The state-of-the-art scaffold-free bioprintings facilitate cellular fusion and maturation through self-assembly, without the assistance of a support structure (i.e. scaffold). Hence, the space for cell growth and ECM formation will not be occupied by the scaffold materials, and the neo-tissue is able to generate directly without the need to consider material degradation. Norotte et al. (2009) used a scaffold-free bioprinting method for small diameter vascular reconstruction. Distinct vascular cell types including fibroblasts and smooth muscle cells, were assembled into discrete units, and printed layer-by-layer concomitantly with agarose rods as a moulding template. More recently, Yu et al. (2016) fabricated scaffold-free tissue strands as a bioink for bioprinting technologies, which has self-assemble capabilities. This promising method has been verified in articular cartilage tissue bioprint using cartilage strands as building units. Although these scaffold-based and scaffold-free bioprinting techniques have demonstrated promising potentials in biomedical applications, research of applying them in tendon TE has yet been reported.

In addition, since there is a potentially enormous demand for the TE tendon implants, and most of the current products are still in laboratory stage, the high-throughput fabrication processes are urgent to be established for scaling-up and enabling their future commercialization. Collaborative works, incorporating experts from various domains including engineering, medicine, and biochemistry will further brighten its prospects and boost its availability in serving the healthcare industry.

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Figure 1 (a) Schematic diagram of the tendon structure from collagen fibrils to the entire tendon (Kannus, 2000), (b) Image of collagen fibrils in extensor tendon (20000×) (Herod et al., 2016), (c) Scanning electron microscope (SEM) image of the cross section of a patellar tendon showing collagen fascicles (F) Enclosed in the epitenon-connective tissue complex (Ep, CT, 16×) (Yahia and Drouin, 1988), and (d) SEM image of tendon crimped structures (10000×) (Raspanti et al., 2005).
Figure 2 Schematic diagram of typical approach for tendon TE
Figure 3 (a) Schematic of electrospinning process. (c) E-jetting process in which printed fibers were patterned using a motion stage (Wu et al., 2015b). (e) Device of electrochemical alignment technique including power supply for providing voltage for the electrochemical cell, syringe pump, rotating electrodes wheel and collection spool (Younesi et al., 2014). (g) Collagen extrusion for fibrillogenesis, followed by winding the fibers onto a spool to generate a fascicle-like multiple-fiber structure (Kew et al., 2012). (i) Wet spinning process in which extruded polymers were treated with multiple coagulant baths, and collected after stretching process (Tamura et al., 2002). (k) Schematic of microfiber melt drawing, which was initiated by inserting a needle into the melt and pulling it downward, and fibers were collected by a rotating mandrel (An et al., 2012). (m) Fabrication process of composite living fibers, in which a biocompatible thread was passed through the baths and was collected by the motor, leading to the formation of a cell-laden hydrogel layer on the thread (Akbari et al., 2014). (b, d, f, h, j, l, n) Images of fiber or fiber bundle manufactured using related techniques (Yin et al., 2010, Wu et al., 2015b, Younesi et al., 2014, Kew et al., 2012, Tamura et al., 2002, An et al., 2012, Costa-Almeida et al., 2017).
Figure 4 Examples of scaffolds fabricated by textile processing and combinations of different technologies. (a) Knitting (Chen et al., 2010). (b) Braiding (Cooper et al., 2005). (c) Scaffold fabricated by braiding three yarns which were twisted from electrospun fibers (Laranjeira et al., 2017). (d) Scaffold fabricated by rolling up a knitted mesh around a braided cord (Fan et al., 2009). (e) Scaffold fabricated by weaving the collagen fibers generated by electrochemical alignment technique (Younesi et al., 2014).
Figure 5 Schematic diagrams of the microcrimping method and SEM image of crimped collagen microfibers (a) An array of synthetic collagen fibers were placed parallelly on a polyurethane substrate with pre-strain. (b) A polyurethane buttressed-rectangular membrane fabricated using photolithography technique was pre-extended, and fixed on the collagen fibers. (c) The pre-extended substrate and membrane was released to generate microcrimp, and transferred to -80 ºC. (d) The membrane was removed, and the frozen fiber array was placed in glutaraldehyde vapor at room temperature for 24h. (e) A solution of elastin-mimetic protein was applied over the microcrimped fiber, and pressed into a thin layer with an acrylic sheet. (f) The acrylic and polyurethane surfaces were then detached from the fiber-reinforced layer. (g) SEM image of fibers crimped using 40% pre-extension (Scale bar = 200 µm) (Caves et al., 2010)
Figure 6  (a) Optical image of the e-jetted pattern with crimped fibers. (b) SEM image of the scaffold after rolling up the mesh in (a). (c, d) CLSM image of nuclei (blue) and F-actin (green) on the scaffold demonstrating their alignment along the crimped fibers (Wu et al., 2017e).
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<td>504</td>
<td>54</td>
<td>15</td>
<td>(Johnson et al., 1994)</td>
</tr>
<tr>
<td></td>
<td>29 - 50</td>
<td>660</td>
<td>65</td>
<td>15</td>
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<tr>
<td>Achilles tendon</td>
<td>35 - 80</td>
<td>820</td>
<td>80</td>
<td>---</td>
<td>(Wren et al., 2001)</td>
</tr>
<tr>
<td>Anterior cruciate ligament</td>
<td>48 - 86</td>
<td>65</td>
<td>11</td>
<td>58</td>
<td>(Noyes and Grood, 1976)</td>
</tr>
<tr>
<td></td>
<td>16 - 26</td>
<td>111</td>
<td>26</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>Biceps tendon</td>
<td>41 - 82</td>
<td>421</td>
<td>33</td>
<td>23</td>
<td>(McGough et al., 1996)</td>
</tr>
<tr>
<td></td>
<td>70 ± 6</td>
<td>400</td>
<td>---</td>
<td>20</td>
<td>(Reuther et al., 2013)</td>
</tr>
<tr>
<td>Wrist extensor tendon</td>
<td>41 ± 12</td>
<td>84</td>
<td>19</td>
<td>---</td>
<td>(Breault- Janicki et al., 1998)</td>
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<tr>
<td>Considerations</td>
<td>Requirements</td>
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<tr>
<td>Biomimicry of fibrous structures</td>
<td>Recapitulate the hierarchical and anisotropic fibrous architecture of tendon tissues</td>
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<tr>
<td>Mechanical properties</td>
<td>Support the load-bearing function of tendon; Sufficient mechanical properties during \textit{in vitro} culturing, and the strength to resist the physiological mechanical environment at the implantation site \textit{in vivo}</td>
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</tr>
<tr>
<td>Porosity and pore interconnectivity</td>
<td>Contain a highly porous and interconnected network that promotes fluid flow, cell infiltration and tissue ingrowth</td>
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<tr>
<td>Pore size</td>
<td>Minimum pore size of 100-250 µm for tissue ingrowth</td>
<td></td>
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<tr>
<td>Collagen fiber crimp</td>
<td>Imitate the crimped morphology of tendon fibers to trigger tri-phase mechanical behaviour and regulate cellular orientation</td>
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<tr>
<td>Biomaterials</td>
<td>Possess sufficient mechanical strength and appropriate degradation characteristic, such as synthetic polymers: PLA, PGA, PLGA and PCL, and natural materials: collagen, alginate, silk, chitosan and hyaluronan</td>
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</tr>
<tr>
<td>Cell source</td>
<td>Maintain tendon phenotypes or facilitate tenogenesis, such as tendon related cell types: tenocytes and tenoblasts, and stem cells: MSCs, ESCs, iPSCs, hTSPCs and ASCs</td>
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<tr>
<td>Cell alignment</td>
<td>Facilitate cellular alignment to promote tenogenesis</td>
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</tbody>
</table>
### Table 3: Summaries of the fiber-based technologies and properties of the fabricated scaffolds for tendon TE

<table>
<thead>
<tr>
<th>Technologies</th>
<th>Scaffold composition</th>
<th>Cell type</th>
<th>Animal type</th>
<th>Fiber diameter</th>
<th>Scaffold Young’s modulus (MPa)</th>
<th>Scaffold Ultimate Strength (MPa)</th>
<th>In vitro</th>
<th>In vivo</th>
<th>References</th>
</tr>
</thead>
</table>
| Electrospinning | PLGA | Human rotator cuff fibroblast | --- | Aligned: ~615 nm Random: ~568 nm | Aligned: ~341 | Aligned: ~12 | • Cells and collagen aligned on aligned fibers  
• Higher α2 expressed on aligned fibers  
• Higher mechanical properties maintained on aligned fibers | --- | (Moffat et al., 2008) |
| Electrospinning | PLGA | Rat tendon fibroblast | --- | --- | Aligned: ~75 Random: ~30 | Aligned: ~45 Random: ~20 | • Expressed COL-I oriented along the aligned fibers | --- | (Xie et al., 2010) |
| Electrospinning | PLA | hTSPCs | Mice | Aligned: ~430 nm Random: ~450 nm | Aligned: ~23 Random: ~0.6 | --- | • iTSPCs oriented on aligned fibers  
• Tenogenic expressions were higher in aligned fibers.  
• Aligned fibers hindered osteogenesis  
• Higher integrin α1, α5 and β1, and myosin II B levels on aligned cells | --- | (Yin et al., 2010) |
| Electrospinning | PLLDLA | Bovine fibroblasts | --- | ~880 nm | ~3 | --- | • Mechanically stimulated crimped scaffold increased collagen and/or PG synthesis  
• Cells seeded on dynamically stimulated crimped scaffolds resembled fascicles | --- | (Surrao et al., 2012) |
<p>| Electrospinning | PLGA | Human rotator cuff fibroblast | --- | 320 nm, 680 nm and 1.80 mm | ~421-510 | ~13 | • Higher cell number, total collagen, and PG production were found on the nanofiber scaffolds | --- | (Erisken et al., 2012) |</p>
<table>
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<tr>
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</thead>
<tbody>
<tr>
<td>Electrospinning</td>
<td>PCL+Chitosan +Cellulose nanocrystals</td>
<td>Human tendon cells (hTDCs)</td>
<td>---</td>
<td>~230-260 nm</td>
<td>~230-540</td>
<td>~21-39</td>
<td>• Aligned fibers promoted cell orientation and elongation</td>
<td>---</td>
<td>(Domingues et al., 2016)</td>
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<td></td>
<td></td>
<td></td>
<td>---</td>
<td>~1.5</td>
<td>~1.45</td>
<td>• Cells were impregnated into scaffold and aligned along fibers</td>
<td>• Cells adopted tendon cell phenotype upon treatment with TGF-β3</td>
<td>---</td>
<td>(Yang et al., 2016)</td>
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<td></td>
<td>PCL+Gelatin</td>
<td>hASCs</td>
<td>---</td>
<td>---</td>
<td>~0.6-1.5</td>
<td>~0.2-0.8</td>
<td>---</td>
<td>---</td>
<td>(Wu et al., 2017a)</td>
</tr>
<tr>
<td></td>
<td>PCL+Hydroxyapatite</td>
<td>---</td>
<td>---</td>
<td>~280-500 nm</td>
<td>~0.6-1.5</td>
<td>~0.2-0.8</td>
<td>---</td>
<td>---</td>
<td>(Wu et al., 2017a)</td>
</tr>
</tbody>
</table>

- Microfibers promoted the gene expression of COL-I, III, V, and tenomodulin
- High proliferation rate
- Enhanced cell infiltration
- Better cell attachment and proliferation than PCL scaffolds
- Torn tissues at the tendon-bone insertion site regenerated with the PCL-chitosan scaffolds showed higher strength and stiffness than those repaired by PCL scaffolds
- Enhanced new bone formation, collagen and GAG expression than PCL scaffolds
| Technologies                  | Scaffold composition | Cell type                        | Animal type | Fiber diameter | Young’s modulus (MPa) | Ultimate Strength (MPa) | In vitro                                                                                           | In vivo | References                                                                 |
|------------------------------|----------------------|----------------------------------|-------------|----------------|-----------------------|------------------------|--------------------------------------------------------------------------------------------------------------------------------|
| **E-jetting**                 | PCL                  | Human tenocytes                  | ---         | ~20 µm         | ~230                  | ~50                    | • Enhanced cell metabolism and migration than electrospun scaffolds                                                                 | ---     | (Wu et al., 2016a, Wu et al., 2017c)                                      |
| **Electrochemical Alignment** | Collagen             | Tendon-derived fibroblast cells  | ---         | ~50-400 µm     | ~277-671              | ~24-88                 | • Cells migrated, proliferated and oriented within 3D constructs formed by multiple bundles.                                                                 | ---     | (Cheng et al., 2008)                                                      |
| **Fiber Extrusion**          | Collagen + PEG       | Ovine tendon fibroblasts and human white blood cells | ~60 µm     | ~27-76         | ~4.6-16.9             |                        | • Cells readily infiltrated the matrix and adhered                                                                 | ---     | (Kew et al., 2012)                                                       |
| **Wet Spinning**             | Silk + Collagen      | Rabbit                            | ---         | ---            | ~9-295                |                        | • Greater cell adhesion and DNA content compared to those on chitosan fibers                                                                 | ---     | (Funakoshi et al., 2005b)                                                 |
|                             | Chitosan + Hyaluronan| Rabbit tendon fibroblast          | ---         | ~30 µm         | ---                   |                        | • COL-I predominated in the hybrid scaffolds, and mRNA level of COL-I in the hybrid scaffolds were significantly greater than that in the chitosan scaffold | ---     | (Funakoshi et al., 2005b)                                                 |
|                             | Alginate + Chitosan  | Rabbit tendon fibroblast          | ---         | ~100 µm        | ---                   |                        | • Alginate-based chitosan fibers showed improved cell adhesion capacity with                                                                 | ---     | (Majima et al., 2005)                                                     |

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<th>In vitro</th>
<th>In vivo</th>
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</thead>
<tbody>
<tr>
<td>Microfiber Melt Drawing</td>
<td>PCL</td>
<td>Human dermal fibroblasts</td>
<td>Rabbit</td>
<td>~10-25 µm</td>
<td>---</td>
<td>---</td>
<td>• Dense COL-I fibers were observed in the hybrid polymer fibers</td>
<td>• Microfibers were highly infiltrated by tendon tissue as early as in 1 month • Repaired tendon had a well-aligned tissue structure</td>
<td>(An et al., 2012)</td>
</tr>
<tr>
<td>Composite Living Fibers</td>
<td>Surgical suturing threads + Alginate + Gelatin</td>
<td>hTDCs</td>
<td>---</td>
<td>---</td>
<td>~6000-8000</td>
<td>~1200-1800</td>
<td>• Encapsulated cells migrated within the hydrogel and aligned at the surface of the core thread • Up-regulated genes expression of scleraxis and tenascin-C, matrix metalloproteinases 1 and 2 • Cells were able to produce a collagen-rich matrix, which is structurally comparable to native tendon tissue</td>
<td>---</td>
<td>(Costa-Almeida et al., 2017)</td>
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<tr>
<td>Technologies</td>
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<tr>
<td>Knitting</td>
<td>Silk + collagen</td>
<td>BMSC</td>
<td>Mouse and rabbit</td>
<td>Silk: ~10 µm Collagen: ~66 nm</td>
<td>---</td>
<td>---</td>
<td>• Cells cultured on a collagen film expressed COL-I and decorin at higher levels than a silk film</td>
<td>• The silk scaffold elicited little inflammation and vascularization, and hardly degraded after implantation for 1 year in a mouse model</td>
<td>(Chen et al., 2008)</td>
</tr>
<tr>
<td>Silk + collagen</td>
<td>hESC-MSCs</td>
<td>Mouse</td>
<td>Silk: ~10 µm Collagen: ~30 nm</td>
<td>~24-34</td>
<td>~4.6-6.7</td>
<td>• hESC-MSCs exhibited spindle-shaped morphology and expressed COL-I, III, Epha4 and Scleraxis, as well as cilia, integrins and myosin under mechanical stimuli</td>
<td>• More regularly aligned cells and larger collagen fibers were observed under in vivo mechanical stimuli</td>
<td>(Chen et al., 2010)</td>
<td></td>
</tr>
<tr>
<td>Silk + collagen</td>
<td>TSPCs</td>
<td>Rabbit</td>
<td>---</td>
<td>~25</td>
<td>~4</td>
<td>• Cells displayed spindle-shape and aligned as early as 24h and deposited ECM at day 7</td>
<td>• More organized regenerative tissue was found on aligned collagen/silk scaffolds than sponge collagen/silk scaffold</td>
<td>(Zheng et al., 2017)</td>
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</tbody>
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<table>
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<tr>
<th>Technologies</th>
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<th>In vivo</th>
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<tbody>
<tr>
<td>Braiding</td>
<td>PGA/PLGA/P LA</td>
<td>Rabbit ACL cells</td>
<td>---</td>
<td>~15-25 µm</td>
<td>---</td>
<td>~117-378</td>
<td>• Rapid degradation of PGA resulted in matrix disruption and cell death</td>
<td></td>
<td>(Lu et al., 2005)</td>
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<td></td>
<td>• PLA scaffolds maintained their integrity and showed superior mechanical properties</td>
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<td>• Fibronectin pre-coated PLA and PLGA scaffolds showed improved cell attachment efficiency and effected the long-term matrix production</td>
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<tr>
<td></td>
<td></td>
<td>Rabbit ACL cells/mouse fibroblasts</td>
<td>---</td>
<td>~15-25 µm</td>
<td>---</td>
<td>~5.3-439</td>
<td>• Scaffolds supported attachment, spreading, and growth for both cell types</td>
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<td>(Cooper et al., 2005)</td>
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<tr>
<td></td>
<td>PLGA</td>
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<td>• Mouse fibroblasts showed random orientation, while rabbit ACL cells oriented along the fibers</td>
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<tr>
<td></td>
<td>Silk</td>
<td>Rat tenocytes</td>
<td>Rabbit</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>• Silk fibroin promoted cell adhesion, propagation and ECM production</td>
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<td>(Fang et al., 2009)</td>
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<td></td>
<td></td>
<td>• Neo-tendon formed after 16 weeks of implantation with uniform and oriented collagen fiber bundles</td>
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<td>• COL-1 dominated during the neo-tendon generation</td>
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<td>• The maximum load of regenerated tendon at 16 weeks reached 55.46% of the normal tendon values</td>
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<td>Technologies</td>
<td>Scaffold composition</td>
<td>Cell type</td>
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<td>Scaffold Ultimate Strength (MPa)</td>
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<td>In vivo</td>
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<tr>
<td><strong>Silk</strong></td>
<td></td>
<td>Human foreskin fibroblasts (HFFs)</td>
<td></td>
<td>~10 µm</td>
<td>---</td>
<td>---</td>
<td>• Cyclic loading decreased the tensile strength of the constructs, with the scaffolds both elongating and stiffening</td>
<td>---</td>
<td>(Li and Snedeker, 2013)</td>
</tr>
<tr>
<td>Braiding</td>
<td>PCL+PLA</td>
<td>hiPSC-MSCs</td>
<td></td>
<td>PLA: ~990 nm</td>
<td>~5-121</td>
<td>~3-50</td>
<td>• hiPSC-MSCs were better attached on PLLA scaffolds compared to PCL scaffolds</td>
<td></td>
<td>(Czaplewski et al., 2014)</td>
</tr>
<tr>
<td>Electrospinning + Knitting</td>
<td>PLGA + Silk</td>
<td>Porcine BMSC</td>
<td></td>
<td>~10-25 µm</td>
<td>---</td>
<td>---</td>
<td>• Cell proliferation on scaffolds with electrospun coating was faster than scaffolds without electrospun</td>
<td></td>
<td>(Sahoo et al., 2006)</td>
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<tr>
<td>Technologies</td>
<td>Scaffold composition</td>
<td>Cell type</td>
<td>Animal type</td>
<td>Fiber diameter</td>
<td>Young’s modulus (MPa)</td>
<td>Ultimate Strength (MPa)</td>
<td>In vitro</td>
<td>In vivo</td>
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<tr>
<td>Electrospinning + Braiding</td>
<td>PLA</td>
<td>hMSCs</td>
<td>---</td>
<td>~700 nm</td>
<td>~50</td>
<td>~7</td>
<td>• Cells showed more functionalized on scaffolds with electrospun coating, as evidenced by the higher expression of COL-I, decorin, and biglycan genes</td>
<td>---</td>
<td>(Barber et al., 2011)</td>
</tr>
<tr>
<td>Electrospinning + Braiding</td>
<td>PCL, PLA</td>
<td>MSCs</td>
<td>---</td>
<td>~900 nm</td>
<td>---</td>
<td>---</td>
<td>• Cells and cytoskeleton displayed alignment on the scaffolds</td>
<td>---</td>
<td>(Rothrauff et al., 2017)</td>
</tr>
<tr>
<td>Electrospinning + Braiding</td>
<td>PCL+chitosan +cellulose nanocrystals</td>
<td>hTDCs and hASCs</td>
<td>---</td>
<td>~92-95 nm</td>
<td>~355-371</td>
<td>~56-61</td>
<td>• Scaffolds induced a high cytoskeleton elongation and anisotropic organization</td>
<td>---</td>
<td>(Laranjeira et al., 2017)</td>
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<tr>
<td>Technologies</td>
<td>Scaffold composition</td>
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<td>Animal type</td>
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</tbody>
</table>
| Wet spinning + Braiding | Chitosan + Hyaluronan | Rabbit tenon fibroblast | Rabbit      | ---            | ~50-90                        | ~5-10                          | ---                                      | • COL-I and crimp were only seen in the cell-seeded scaffold and increased in the regenerated tissue  
  • The tensile strength and modulus in cell-seeded scaffold improved from 4 to 12 weeks postoperatively  
  • The cell-seeded scaffold had a greater modulus than non-cell-seeded scaffold at 12 weeks | (Funakoshi et al., 2005a) |
| Knitting + Braiding | Silk                 | MSCs               | Pig         | ~8 µm          | ---                           | ---                            | • MSCs proliferated and differentiated into fibroblast-like cells by expressing COL-I, III and tenascin-C genes on the scaffolds  
  • The scaffold was filled with abundant newly formed fibrous tissue, and surface was covered by a thin layer of synovium-like tissue after 24 weeks of implantation  
  • The key ligament-specific ECM components were produced and indirect ligament-bone insertion with three zones (bone, Sharp’s fibers and ligament) was observed  
  • Tensile properties of regenerated ligament could be maintained after 24 weeks of implantation with remarkable scaffold degradation  
  • Collagen fibers were arranged in the direction of tensile load. Col-I was strongly positive in newly regenerated tissue, and COL-III and tenascin-C was weak positive | (Fan et al., 2009) |
| Electrochemical Alignment + Weaving | Collagen          | MSCs               | ---         | ~100-150 µm    | ~150-500                      | ~20-65                         | • Cells uniformly distributed within the scaffolds  
  • Cells showed tenogenic | ---                                      | (Younesi et al., 2014) |
<table>
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</table>

- Differentiation in the absence of growth factors
- Matrix which was positive for tenomodulin, cartilage oligomeric matrix protein and COL-I, was synthesized