Chapter

Advanced silk-based biotextiles for bone regeneration applications

Viviana P. Ribeiro[1,2,*], Joaquim M. Oliveira[1,2] and Rui L. Reis[1,2]

*Viviana Pinto Ribeiro, BSc, MSc, PhD (✉)
[1]3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Parque de Ciência e Tecnologia, Zona Industrial da Gandra, 4805-017 Barco, Guimarães, Portugal;

[2]ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, 4805-017, Portugal;

Joaquim Miguel Antunes Correia de Oliveira, BSc, PhD • Rui Luís Gonçalves dos Reis, BEng, MSc, PhD, DSc, PhD/MD
[1]3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Parque de Ciência e Tecnologia, Zona Industrial da Gandra, 4805-017 Barco, Guimarães, Portugal;

[2]ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, 4805-017, Portugal;

*Corresponding author
e-mail: viviana.ribeiro@i3bs.uminho.pt

Abstract Increasing efforts have been made in tissue engineering (TE) research for novel biomaterials and scaffolds that can efficiently support bone tissue regeneration and repair. Textile-based technologies are predefined manufacturing processes of particular interest since they allow for producing finely tuned fiber-based structures with controlled three-dimensional architecture and improved mechanical properties. Highly reproducible scaffolds can be achieved with interconnected macro- and micro-porosity suitable for controlling cell functions and guiding bone tissue regeneration and repair. Herein, the recent studies dealing with the processing methodologies, physical properties, and biocompatibility of fiber-based scaffolds for bone TE applications are overviewed. The fundamentals and application of silk fibroin (SF) protein as biomaterial for scaffolds production, made up of micro- and nano-fibers are also considered. The promising outcomes of such investigations are summarized and discussed in depth.

1. Introduction

Tissue engineering (TE) and regenerative medicine approaches focus on restoring (form and function) tissue deformities [1]. Different tissues, like cartilage [2], nerves [3], ligament [4], skin and vessels [5] have been engineered through innovative TE approaches. Bone is a dynamic and complex tissue that provides stiffness and structural support to the body. It is highly vascularised and holds a remarkable intrinsic ability to remodel [6]. For large bone defects related to trauma, congenital malformations or surgical resection, the critically sized osseous deficiency is particularly important, since it does not regenerate spontaneously. Thus, the regeneration of such defects has been inspiring for the research of novel and more effective bone TE strategies [1]. Some considerations are required in the development of scaffolds for bone regeneration. They need to facilitate and promote cell adhesion, proliferation and neo-tissue formation/integration within the material, without inducing any inflammatory response. The intra-architectural scaffold geometry, porosity, scaffold material and surface area play important roles in this process. Structures with high macro-/micro-porosity and large surface area will allow for cell ingrowth, distribution and neovascularisation [7, 8]. With this in consideration, major progresses were done in the past years with numerous approaches to design the "ideal" scaffold for bone TE and that meet all the requirements for effective tissue regeneration.

Fiber-based technologies have been shown interesting properties to prepare polymeric-based architectures for bone TE [9, 10]. It was back in the 1990's that the term "biotextile" was defined as a "structure composed of textile fibers and designed for use in a specific biological environment (e.g. surgical implants)" [11]. More recently, textile-based technologies were recognized in TE field as a new route for producing more complex three-dimensional (3D) fibrous structures. Through these fully automated technologies, scaffolds production can be performed with improved reproducibility and controlled structural properties [12]. Furthermore, the primary quality requirements for TE approaches, such us, biocompatibility, flexibility and strength, can also be found on textile-based scaffolds [13]. Flock, stitching, braided, woven, non-woven or knitting are some of the textile-based technologies already proposed for TE [14-16] and bone TE applications [7, 15, 17]. Among the long list of textile materials, spacer fabrics increased their popularity in some biomedical areas due to their ability to significantly increase the scaffolds three-dimensionality [18]. For bone TE applications this is a desired technology since the resulting scaffolds may present superior internal porosity, ideal for ECM formation and vascularization, high resistance to deformation and similar anisotropic properties to bone [7].

Different types of natural and synthetic polymers have been investigated as scaffolding materials [19]. Ceramic implants made of hydroxyapatite (HA) or other calcium phosphates (CaPs) are still

the most desirable due to the chemical similarities to bone tissue. At the same time, natural-origin materials and proteins were recognized for their biocompatibility, biodegradation and osteoconductivity [1, 20].
Silk fibroin (SF) can be obtained from several arthropods presenting different conformational structure and biodegradability. SF-based structures can offer impressive mechanical properties, biocompatibility, environment stability and versatility, which fulfills the requirements for an effective tissue regeneration [21]. The mechanical strength and elasticity are important advantages of silk compared to other natural polymers, not to mention the presence of easy accessible functional groups for chemical modifications [22]. SF derived from silkworms has a long history of using as textiles for biomedical applications (sutures and bandages), nevertheless only recently textile-based SF materials has been explored for TE [14, 23] and bone TE applications [7]. Some of these SF-based textile scaffolds were implantable and shown biocompatibility and sensitivity for advanced *in vivo* applications. These recent studies are leading to promising approaches of using SF as biomaterial for bone TE applications. This chapter is focused on recent and relevant research based on SF applied for bone regeneration, involving fiber- and textile-based technologies for scaffolds processing. Additionally, the different platforms are discussed according to the bone TE strategy.

2. Concepts in bone biology

Bone tissue is considered a complex system responsible for providing human body support and protection to organs. It is responsible for maintaining homeostasis and participates on blood cells formation. Approximately 70% of bone is inorganically constituted, while the remaining 30% are organic matter [24]. Collagen is the main constituent of the organic extracellular matrix (90-95%), along with other self-assembled glycoproteins, such us, osteocalcin (OCN) and osteopontin (OPN), bone sialoproteins (BSP), and proteoglycans. The inorganic mineral component of bone is constituted by HA nanoparticles and inorganic salts that within the collagen fibrils act as a reinforcement system of the tissue. Moreover, the synthesis of alkaline phosphate (ALP), which provides a direct index for bone formation rate, is upregulated by this system. The Ca/P ratio of HA is 1.67 and the nanosize of its particles favours cell proliferation within the bone [24, 25]. Thus, the development of artificial biological active HA nanoparticles should mimic such requirements for an effective biological and mechanical response. Different types of cells interact for the maintenance of a healthy bone tissue and all of them have defined activities [24]. Osteoblasts are derived from mesenchymal stem cells (MSCs) located in the bone marrow and are responsible for bone formation and extracellular matrix (ECM) synthesis. When osteoblasts are embedded in their own matrix become osteocytes, the highest differentiation state of these cells and responsible for bone maintenance. Osteoclasts are multinucleated giant cells originated from hematopoietic stem cells and responsible for bone resorption. This process occurs due to the self-healing capacity of bone tissue initiated by the digestion of the HA nanocrystals and collagen fibrils at the defect site [26]. Osteoclasts have been hypothesized to play an important role in response to mechanical stimuli, harbouring the intrinsic plasticity of bone [27]. The bone cell types are present in different spaces within bone tissue, which in adults is divided into cortical or compact and trabecular or spongy bone [28]. Cortical bone contains the osteocytes in the lacunae of osteons and blood vessels that run parallel to the bone's long axis, while the trabecular bone is highly porous, do not contain Haversian system (osteons) and holds the red bone marrow [29].
Even though bone has self-healing capacity, this might be compromised by the extension of the lesions. For that, bone TE came to respond to such needs especially when the well-established

medical implants are not a viable solution. Bone TE relies on a series of knowledge about bone biology, structure and mechanical composition. More importantly how these systems are interconnected for a proper tissue function and remodelling. For example, stem cells play an important role to trigger bone regeneration process since they are responsible for guiding osteogenic cells lineage and consequently ECM production [30]. The immobilization of growth factors and cytokines is a derivative of cells behaviour creating a natural scaffold network for tissue remodelling [31]. Calcium absorption is also dependent on vitamins (vitamin D) and hormones (parathyroid hormone) that exert a major influence on mineral metabolism and homeostasis [32]. Overall, bone is a highly dynamic tissue and even if all the biological requirements, including cells, ECM, cell-matrix interactions and growth factors, are fulfilled in a TE approach, a 3D configuration is needed so that cells can grow inside and form a 3D matrix that can better mimic bone architecture.

3. Silk fibroin structure and chemical composition

Silk proteins are present in the gland of silkworms, spiders, scorpions, mites or bees, from where can be directly extracted or spun as fibers. Silkworm's silks are well established in the textile industry, and for biomedical applications as sutures and bandages. They can be produced by silkworms of the *Bombycidae* family (mulberry silk), which is the case of *Bombyx mori* silk, or by the *Saturniidae* family (non-mulbarry silk) [33]. The spider's silks present outstanding mechanical properties but are heterogeneous in nature, which limits their use for commercial applications and biomaterial products design [34]. Regardless of their source, the spun silk fibers are composed of a core protein named fibroin evolved by a glue-like protein named sericin that can be removed by alkali- or enzyme-based "degumming" processing. The obtained purified silk fibroin (SF) is the most commonly type of silk used for biomaterials processing representing 70% of the total silk [35, 36]. Silk sericin (SS) represents 30% of the protein and has shown anti-bacterial properties [37, 38]. However, some indications suggest adverse cytotoxic effects induced by this protein [39]. For that reason, the removal of SS is a "standard" procedure in textile industry and for silk-based biomaterials processing. Also, sericin recovery before manufacturing reduces the environmental impact [40].

SF from *Bombyx mori* cocoons has a large molecular weight of 200-350 kDa and is composed by heavy (H) and light (L) chains of anti-parallel β-sheet and amorphous conformation (**Figure 1a**). The H chains contain repetitive hydrophobic domains of Glycine-X (X is Alanine, Serine, Tyrosine, Valine) intercalated by small non-repetitive hydrophilic domains forming β-turns (Lysine, Glutamic acid, Aspartic acid, Arginine) (**Figure 1b**). The L chains are smaller, hydrophilic in nature and its sequence is not involved in the crystalline region of SF, giving a certain elasticity to the protein [33, 41]. The H and L chains are linked together by a single disulfide bond at the C-terminus of the H chains. A small glycoprotein (P25) of 25 kDa is also present in the H-L chain complex (ratio of 6:6:1), forming micellar units and playing an important role in maintain the complex integrity [42]. Non-mulberry SF is exclusively composed of H chains that can reach 395 kDa, and contain higher Alanine/Glycine ratio and poly-alanine blocks, which forms stronger β-sheets. Moreover, this SF has the particularity of contain RGD sequence (arginine-glycine-aspartic acid) making it more available for cell interactions and with superior cell adhesion properties [43, 44].

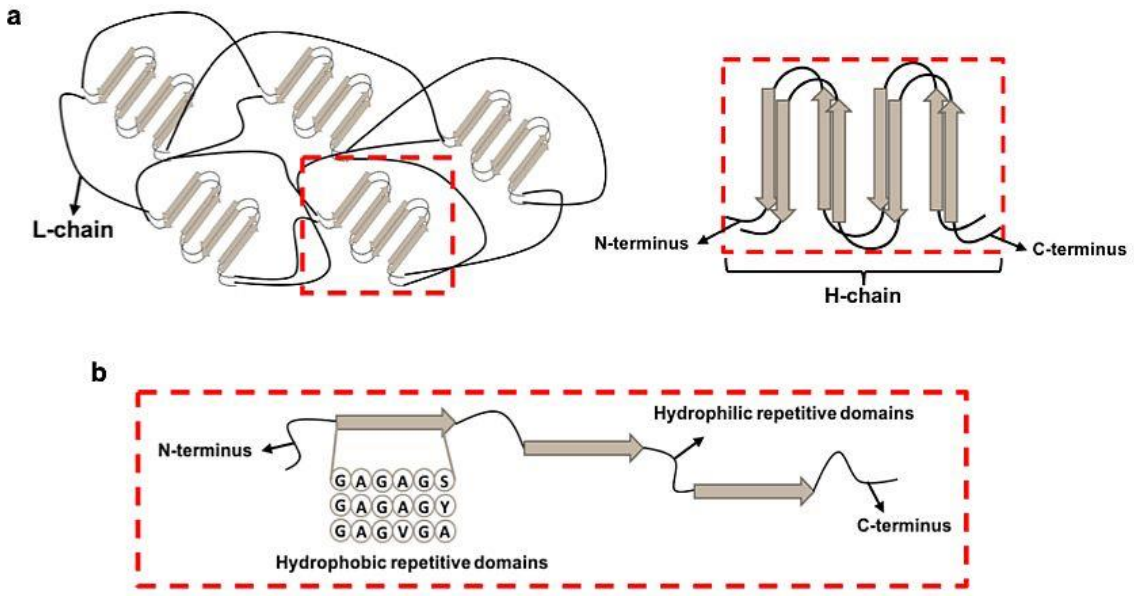


Figure 1. Schematic illustration of silkworm SF fibers: (a) SF composed of heavy chains (H-chain) and light chains (L-chain). (b) Each SF H-chain consists of hydrophobic and hydrophilic repetitive domains.

The final structural conformation and crystallinity of SF-based biomaterials will be determined by the higher or lower content of β-sheets that are formed during protein processing. The nano-crystallinity of SF have shown to induce superior mechanical properties to materials [45]. At the same time, the semi-crystalline and amorphous domains on SF ensure some elasticity and resilience to the structures [45, 46]. An important feature of SF is the possibility of control its size, distribution, orientation and spatial arrangement only by applying different processing techniques and physical/chemical treatments [43]. Thus, the wide range of SF sources allied to the easy processing and chemical manipulation can open for the possibility of creating different biomaterials with specific structural and biological properties.

4. Silk fibroin scaffolds for bone regeneration

The complexity of bone implies for different perspectives when scaffolds are designed for regeneration and tissue repair. The material choice and processing ability are decisive factors in bone TE. Naturally, the chemical composition and high processability of SF inspired materials scientists to produce a wide range of SF-based scaffolds for TE applications. Among them, hydrogels, sponges, 3D printed structures and composite scaffolds are the most relevant for bone TE applications [35]. Hydrogels are water-swollen polymeric networks with structural similarities to the ECM of tissues. For this reason, hydrogel-based matrices have been quite used in TE strategies specially for tissues with high water content, such us, cartilage, meniscus and intravertebral disc [47]. According to bone tissue requirements, water-swelling properties are not requested for scaffolds design. Moreover, the high hydration degree of hydrogels may affect the mechanical properties of matrices, which are critical for bone regeneration and tissue repair. Thus, hydrogels proposed for bone TE may be subjected to a post-hydrogelation processing in order to

induce porosity and improve the final mechanical properties of the structures [48]. The hydrogelation of aqueous SF solutions is induced through different physical and chemical methods, including high temperatures, low pH, ionic strength, vertexing, sonication, freeze gelation, methanol/ethanol treatment, and blending/crosslinking with other polymers [49, 50]. Using these methods, the hydrogelation process may occur by an increase of β-sheets content and SF crystallinity, which physically stabilizes the hydrogels and improves its mechanical properties [51]. Electrogelation and enzyme-mediated hydrogelation are exceptions, as it lead to random coil and α-helices structures rather than β-sheets [45, 46, 52]. In these cases, the SF-based hydrogels can be processed through solvent-casting and/or freeze-drying post-treatments in order to increase the β-sheet content for improving the mechanical properties, and induce controlled porosity and interconnectivity into the forming scaffolds [8, 53, 54]. Such techniques have also been applied as processing methodologies of aqueous SF solutions to form porous sponge-like SF scaffolds [55-57]. Sponges have been widely used in bone TE, presenting a 3D porous structure desired for cell attachment, proliferation and migration, and to facilitate nutrients transport within architectures for ECM formation. Through techniques like gas foaming, melt molding, porogen-leaching, and freeze-drying it is possible to control the size of the porogens according to the type of injury and bone tissue regeneration approach [36]. However, and despite the efforts, these fabrication methods fail to control the internal geometry of the scaffolds presenting in most cases a random porosity and no defined intra-architecture. Thus, the emergence of rapid prototyping technologies, such us, 3D printing has enabled to develop customized SF-based scaffolds with pre-designed intra-architectural properties that favor homogeneous vascular ingrowth and new bone integration within the constructs [58]. The big problem is debated in the attempt to direct print SF hydrogels as a single bioink. As previously mentioned, most of the processing methodologies of SF hydrogels involve the production of crystalline hydrogels with high β-sheet content and low hydration degree. Since water content is crucial for cell encapsulation, this revealed to be problem on using SF hydrogels for 3D bioprinting, despite showing adequate mechanical properties [59]. Thus, authors proposed to use SF hydrogels in a main amorphous conformation for 3D bioprinting strategies which allowed for better printing parameters, suitable for cell encapsulation and with adjustable mechanical properties [60, 61]. Regardless of the technology used for producing SF-based scaffolds for engineering bone tissue, they are often combined with inorganic materials which have shown to be beneficial for osteogenic purposes, namely, CaP-based inorganic components [8, 53-57, 62]. Micro and nano-CaPs can be incorporated into the SF matrices by direct deposition and mechanical agitation of dry powders [8, 53, 54, 62, 63], or by using an *in situ* alternate soaking method of mixing $CaCl_2$ and Na_2HPO_4 with a porogen like NaCl [55-57]. In both cases, SF hydrogel matrices and aqueous SF solutions can be used in combination with the inorganic components. The osteoconductivity of the resulting composite materials can be improved by the osteoinductive properties of CaPs, as well as, the presence of nucleation sites for new bone formation. Moreover, the SF/CaP composites usually present high compressive modulus in order to meet the compressive properties of bone and directly support the load-bearing during tissue regeneration [64].

5. Silk fibroin biotextiles for bone regeneration

Fiber-based networks have been showing tunable surface properties with particular interest for developing scaffolds for tissue engineering (TE) applications [65, 66]. At the same time, textile technologies have been evolving in the medical field, and their application in TE approaches has grown in the recent years [15]. Silkworm's silk is of special interest to prepare fiber-based or

textile-based scaffolds, since the silk fibers can be directly obtained from cocoons. Moreover, this type of silk has been extensively explored in the textile industry and with an extensive knowledge about its processing. This means that there is a steady silk production to serve as raw material platform and very sophisticate textile processing technologies which can be useful to create new biomaterial architectures. These are great advantages of using silkworm's silk as compared to other sources. From the silkworm's silk, high amount of fibers can be yielded from a single cocoon (600–1500 m), as compared to the 137 m that can be obtained from the spider's glands or 12 m from the spider's web [67, 68]. Moreover, the easy and non-invasive process of silk extraction together with the homogeneous behavior of the silkworms during their lifetime also influence their choice [33].

5.1. Nanofiber-based scaffolds

Fibrous scaffolds can be obtained by different processing technologies, including electrospinning, phase separation, and molecular self-assembly [69]. Electrospinning technology has been well explored in TE field as a potential mean of scaffolds production, allowing the processing of nanofibers with interconnected and a pre-defined pore structure, as well as, superior mechanical properties [70]. Another interesting property of this technology is the simplicity and low cost setup, involving a typical syringe pump, high voltage source, and a collector. During the extrusion process a polymer solution is charged into the syringe and extruded from the needle by surface tension [71]. Different studies explored the properties of SF as an extrudable material for bone scaffolds production. Kim et al. [72] observed that electrospun SF nanofiber membranes presented favorable biological properties and induced bone regeneration on critical-size rabbit defects. *In vitro* tests showed that pre-osteoblast cells presented a stellate shape and broad cytoplasmatic extensions along the membranes (**Figure 2**). Moreover, high levels of OCN and ALP as specific products of osteoblasts, as well as, ECM calcification were observed. From *in vivo* results, a complete healing and new bone formation was observed after 12 weeks of implantation. The nanoscale benefits of electrospinning processing were confirmed by Jin et al. [73], showing that electrospun SF-based fibers with average diameters of 700 ± 50 nm provided superior cell behavior due to the high surface area. Regenerated aqueous SF solutions were electrospun in combination with poly(ethyleneoxide) (PEO) to allow a stable spinning process. Human marrow stromal cells (BMSCs) showed extensive proliferation and matrix coverage on electrospun SF mats with extracted PEO, suggesting the potential use of these matrices as scaffolds for bone TE. In a different study, Meechaisue et al. [74] was able to fabricate ultra-fine SF-based fibers also using aqueous SF solution at different concentrations and applied in an electrostatic field for the spinning process. The average diameter of the e-spun fibers was dependent on the SF concentration and source, ranging from 183 and 810 nm. The potential use of these SF fiber mats for bone tissue culture was assessed using osteoblast-like cells that were able to adhere and proliferate on their surfaces.

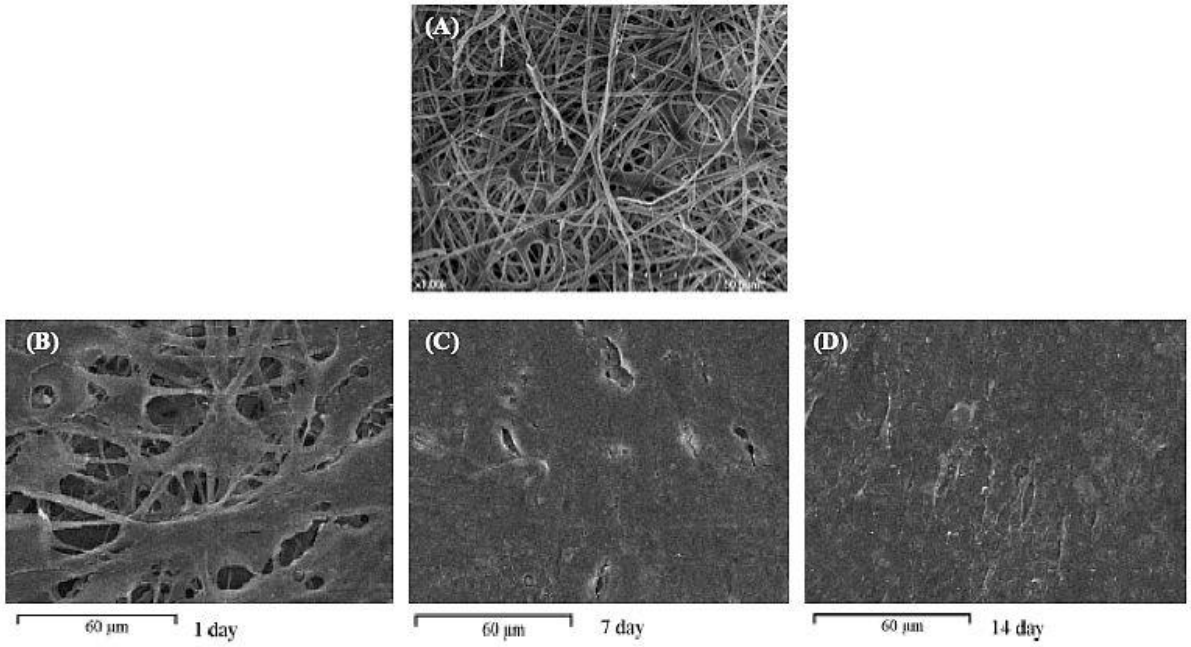


Figure 2. Scanning electron microscopy (SEM) images of (A) electrospun SF nanofiber membranes, and (B-D) MC3T3-E1 cells grown on their surface. Original magnification: 1000x. Adapted from [72] with permission.

In more complex approaches, several authors proposed electrospun SF-based composite scaffolds containing nano-hydroxyapatite (nHA) [75-78], nano-CaPs [79], and/or bone morphogenetic protein-2 (BMP-2) [76, 77, 80, 81] that were included in the aqueous-based electrospinning processes to mimic the natural bone tissue architecture. The functional fibrous substrates were able to support cell attachment, proliferation and bone marrow-derived mesenchymal stem cells (BMSCs) differentiation. Moreover, BMP-2 bioactivity supported higher calcium deposition and enhanced transcript levels of bone-specific markers, while the inorganic nanoparticles improved the mechanical properties of the composites and induced ECM mineralization relevant for new bone formation. The BMP-2 polypeptide has also been included in SF electrospun scaffolds by grafting onto graphene oxide (GO) (which is rich in functional groups) and then bounded to the SF nanofibers through electrostatic interactions [82]. The main purpose of this study was to improve the biocompatibility of the functionalized SF scaffolds, which showed enhanced osteogenic differentiation of BMSCs and significantly promoted *in vivo* new bone formation in critical-sized calvarial bone defects. The enhancement of bone formation promoted by electrospun poly(L-lactic-co-glycolic acid) (PLGA)-tussah SF (TSF) nanofiber scaffolds incorporating GO was also shown by Shao and colleagues [83]. Herein, the blending of TSF into PLGA nanofibers significantly decreased the fibers diameter and improved the mechanical properties of the composites, boosted by the GO inclusion. Cell adhesion, proliferation and osteogenic differentiation of mesenchymal stem cells benefit from scaffolds new properties (**Figure 3**), as well as, the biomineralization-relevant ALP activity and mineral deposition. Xing et al. [25] proposed the electrospinning processing technology to produce SF-based scaffolds containing magnesium oxide (MgO) nanoparticles for accelerating bone regeneration. Nano-MgO was incorporated into electrospun SF blended with polycaprolactone (PCL), showing that the SF/PCL fibrous scaffolds displayed a controlled release of Mg^{2+} resulting in a significant enhancement of

bone regeneration after *in vivo* implantation in critical-sized calvarial defects. Not only synthetic polymers have been blended with SF for creating fiber-based structures for bone TE. Natural-origin polymers, e.g. chitosan or cellulose, have shown interesting properties as electrospun nanofibrous composite materials [84, 85]. Blending SF with such materials improved the mechanical properties and the osteogenic potential of scaffolds, while taking advantage of the biocompatibility and biodegradability of each individual component.

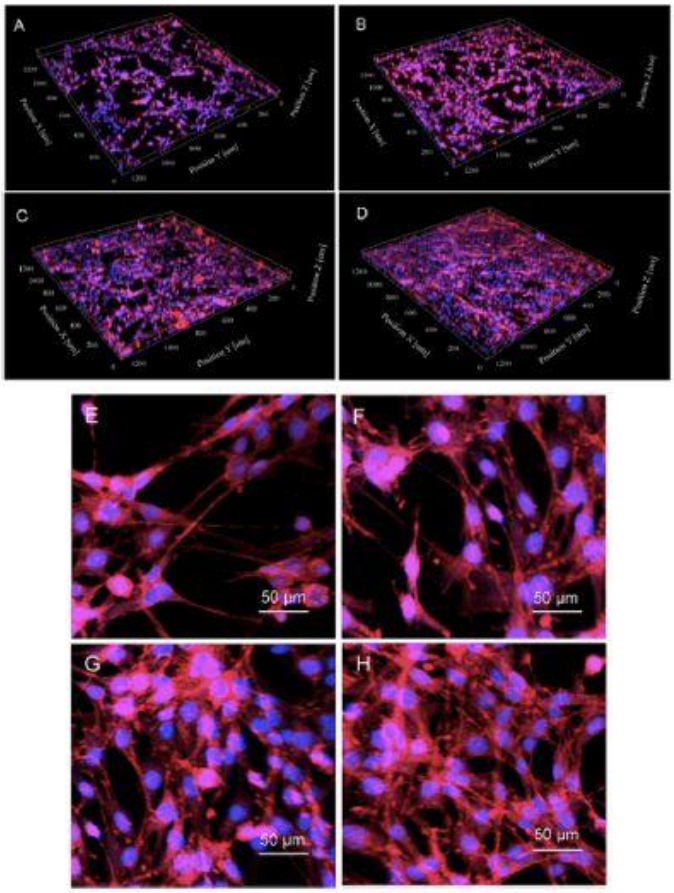


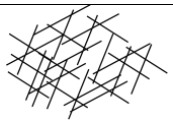
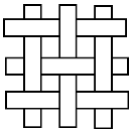
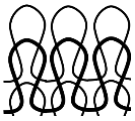
Figure 3. Confocal fluorescent microscopic images of mesenchymal stem cells cultured on different substrates after 7 days: (A, E) cover slip, (B, F) PLGA fibers, (C, G) PLGA–tussah SF fibers, and (D, H) GO-doped PLGA–tussah SF fibers. Reprinted from [83] with permission.

5.2. Textile-based 3D architectures

It is well recognized that a 3D structure is fundamental for an adequate regenerative process that is initiated by cell adhesion, ingrowth and reorganization of the newly formed ECM. The development of porous structures with sufficient mechanical properties will provide the necessary space for *in vivo* neovascularization of tissues like bone [1, 86]. Thus, fibers can be processed to form more complex 3D structures using textile-based technologies. Knitted, braided, woven and non-woven are textile-based technologies already proposed for biological applications [87, 88]. The orientation of the fibers in these structures can vary from highly regular to completely random, but always responding to a pre-designed manufacturing process that ensure highly reproducible structures. The general properties of these textile-based technologies are summarized in **Table 1**.

Woven textiles have a stable and porous-like structure but they can be misaligned at the edges when are cut obliquely for implantation. Braided structures are known as suture makers, and can be designed using several patterns. Moreover, the spaces between the yarns make them porous and help the fluids to flow during the healing process. The non-woven textiles are of random porosity, present isotropic structure, and good ductility, which provides good mechanical properties and thermal stability desired for many TE applications [87]. Finally, the knit structures are particularly useful for developing scaffolds for bone TE applications, since they allow for producing structures with better extensibility and higher porosity/volume. In the knitting process, the needles form symmetric and continuous series of inter-looping stitches that connected together form the final fabric [89]. In these structures, the rows formed across the width of the fabric are called courses, and the columns designed along the length of the fabric are known as wales [90]. Depending on the direction of the formed loops, the knitted fabrics can be classified weft-knitted and warp-knitted fabrics. In the weft-knitting, the wales are perpendicular to the course of the yarn, whereas in the warp-knitting the wales run parallel to the courses [89, 90]. More importantly, is that the entire fabric can be fabricated from one single yarn, in contrast to the warp-knitted structures that require a different yarn in each wale.

Table 1. General properties of textile-based technologies.

Technology	Source	Porosity	Mechanical behavior	Hierarchy	Ref.
Non-woven	Fibers	Varying	Isotropic		[87]
Woven	Yarns	Low	Anisotropic		[91]
Weft-knitted	Yarns	High	Varying from anisotropic to isotropic		[89, 90]
Warp-knitted	Yarns	High			
Braided	Yarns	High	Anisotropic		[88]

SF has already been proposed as a viable multifilament material for scaffolds production [92]. Its natural fibrous structure have shown to be advantageous for the reinforcement of scaffolds designed as bone graft substitutes [93]. The addition of purified and unprocessed SF non-woven fibers into SF freeze-dried scaffolds enable to increase the scaffolds compressive modulus, while maintaining high porosity and slower degradation rates. The subsequent deposition of HA

nanocrystals created an additional response of osteogenic-related markers (i.e. collagen-I, OCN, OPN, and BSP) substantiating the applicability of the tricomposite scaffolds for bone-related applications. Pankaew and White [94], crystallized non-woven SF nets and woven SF fabrics with calcium deficient nano-HA (Ca-def HA) showing that in both cases the nanocrystals were successfully deposited in the fibers improving the mechanical properties and the surface area of the fabrics for potential bone regeneration applications.

The first and only SF-derived scaffold commercially available is made of patterned knitted fibers that showed to be mechanically resistant and flexible for soft-tissues support [95]. In a work developed by our group, Almeida et al. [14] proposed a weft-knitting technology to process SF fibers yielded from silkworm cocoons as scaffolds for TE applications (**Figure 4**), showing that the proposed textile technology offer a superior control over scaffolds design (e.g. size, shape, porosity and fiber alignment), manufacturing and reproducibility. The pre-defined intra-architectural geometry of the fabrics allowed cell attachment, proliferation and deeply infiltration within the porous structure. Moreover, a high porosity and interconnectivity were achieved known for improving tissue infiltration and neovascularization. In the same study, the synthetic and biodegradable polymer polybutylene succinate (PBS) was also proposed as multifilament fibers to produce knitted textile scaffolds, showing similar morphology and biological behavior to that observed on the weft-knitted SF structures. On the other hand, SF matrices presented a considerable higher strength and stiffness as compared to PBS which was expected considering the extraordinary mechanical properties of SF that rival most high performance of synthetic fibers, and is of extreme importance for the mechanical support of hard tissue like bone. Given the processing efficacy and versatility of the proposed knitting technology, the authors considered this system attractive for the functional engineering of different tissues, including bone or cartilage.

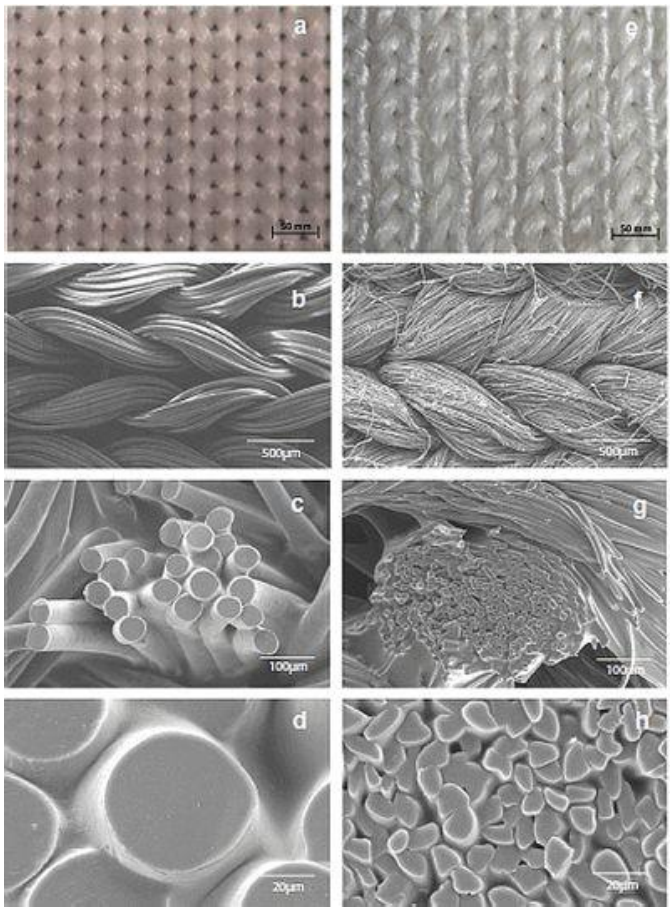


Figure 4. Morphology of (a–d) PBS and (e–h) SF knitted constructs showing different levels of detail. (a, e) Macroscopic images and (b, f) SEM micrographs showing the top view (c, d, g, h) and the respective fiber cross-sections. Reprinted from [14] with permission.

The same SF knitted matrices were further treated using several surface modifications known for improving the structural, mechanical and even biological properties of materials [23]. Sodium hydroxide (NaOH) solution, ultraviolet radiation exposure in an ozone atmosphere (UV/O_3) and air plasma treatment followed by acrylic acid (AAc), vinyl phosphonic acid (VPA) and vinyl sulfonic acid (VSA) grafting, showed different impacts on the final mechanical performance of the textile scaffolds, especially the UV/O_3, plasma/AAc and plasma/VPA that increased both the strength and ductility of structures. The surface topography and chemical composition assessed by atomic force microscopy and X-ray diffraction confirmed the modification and grafting of the surfaces, although with different efficiencies. When transposed to *in vitro* effects, no significant differences were observed in terms of L929 fibroblast cell adhesion and spreading between the modified and unmodified SF textiles. In fact, a superior cell adhesion and flattened morphology was observed on the fiber-based structures, as compared to SF membranes used as control, suggesting that the weft-knitted SF scaffolds presented *per se* superior biological efficacy.
In these studies [14, 23], the standard weft-knitting technology was with success applied to create SF-based platforms for the functional engineering of bone, showing structures of superior extensibility and compliance. Nevertheless, to fully recreate the complexity and anisotropy of bone tissue, an increase of three-dimensionality is required. With this in consideration, novel biotextile

scaffolds were proposed using two external layers of weft-knitted SF connected by a resilient monofilament of synthetic polyethylene terephthalate (PET) (z direction) (**Figure 5**) [7].

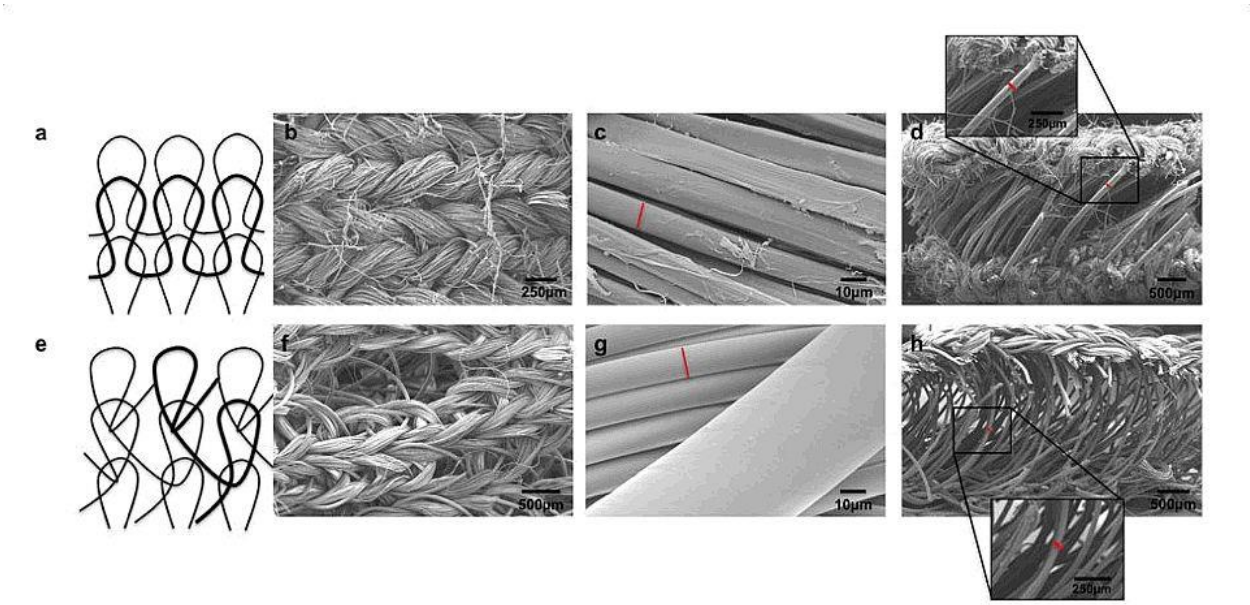


Figure 5. Illustrative images showing the wale and course components of the: (a) weft and (e) warp knitting technologies, used for processing the SF and PET filaments in the scaffolds external layers, respectively. SEM micrographs of (b-d) SF-PET, and (f-h) PET knitted spacer fabrics showing different levels of detail. Morphology of: (b, c) SF and (f, g) PET external layers from a top view perspective, and the respective (d, h) cross-sections showing the resilient PET monofilament (z direction). The red bars indicates: (c) SF and (g) PET fibers diameter (~15 mm); (d,h) PET monofilament diameter (~100 mm) [7].

These fabrics can be created with different choices of yarns to meet a wide range of specifications in the external layers. Moreover, the upper and lower knitted layers are interconnected by a resilient monofilament yarn in the z direction, which represents the adjustable height of the spacer fabric [18]. Monofilament polyester fibers have been specially used for this purpose, holding a marked stiffness that provides higher compression resistance to the fabric. Thus, advanced materials can be reinforced by these textile platforms, especially highly loaded structures. The cellular components can also benefit from these architectures for cell infiltration, ECM formation and bone tissue ingrowth [96]. Focusing on the singular properties of this technology, knitted spacer fabrics were engineered for specific craniofacial bone regeneration applications [7]. Results showed that the novel SF-based 3D biotextiles presented suitable porosity and superior mechanical properties for bone regeneration applications, when compared to warp-knitted spacer scaffolds entirely made of PET. Once again, the extraordinary mechanical properties of SF overlap those of high performance PET fibers [14]. Osteogenic differentiation profile was similar between the two types of constructs. The seeded human adipose-derived stem cells (hACSs) showed the typical peak of ALP activity after 14 days of osteogenic culturing, followed by ECM mineralization. The genotypic and phenotypic expression of osteogenic-related markers also confirmed cell differentiation within the structures after 28 days of osteogenic culture. From *in vivo* studies, superior angiogenic effects were induced by the SF-PET scaffolds when performing a chick chorioallantoic membrane assay. Additionally, both structures allowed tissue ingrowth and blood vessels infiltration after subcutaneous implantation in CD-1 mice (**Figure 6**). All combined, these

features revealed to be important when considering a scaffold for bone defects implantation and tissue regeneration.

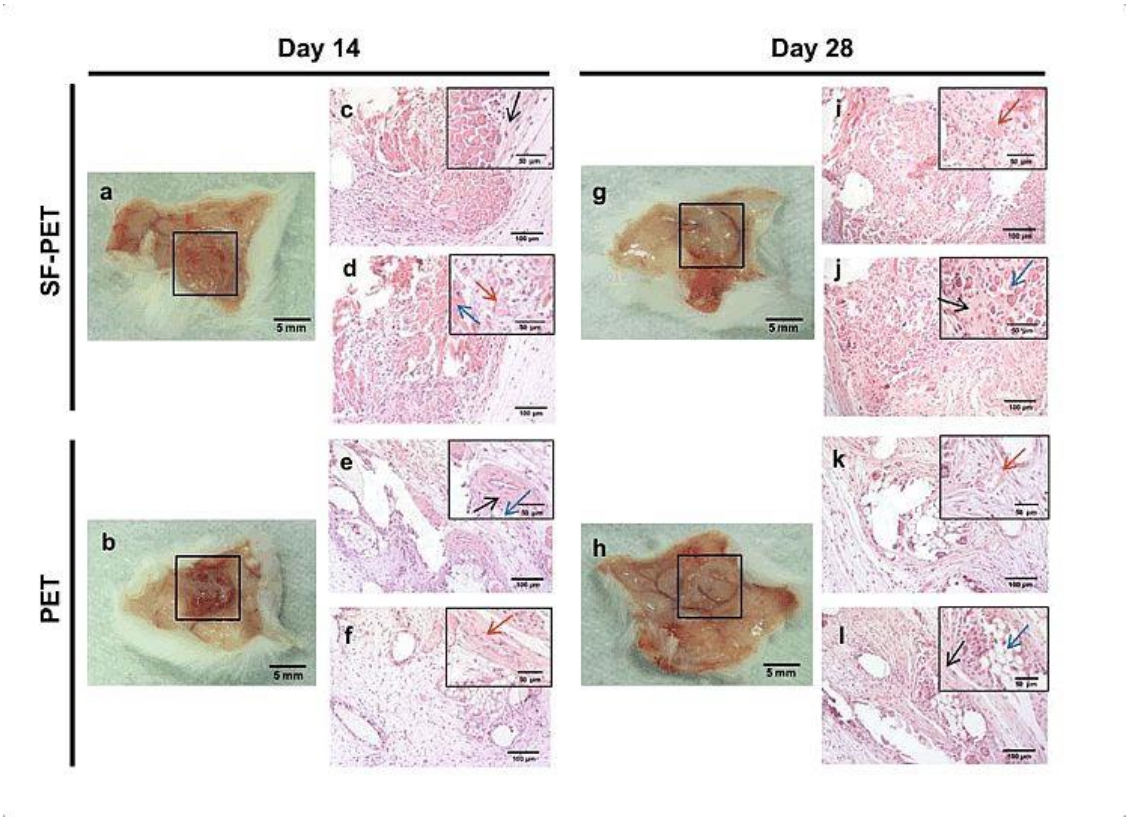


Figure 6. Subcutaneous implantation of the SF-PET and PET knitted spacer fabrics in mice. Macroscopic images of the explants after implantation for (a, b) 14 days, and (g, h) 28 days. H&E staining of the SF-PET (c, d, i, j) and PET (e, f, k, l) explants. Squares represent the regions corresponding to the implanted SF-PET and PET textile scaffolds. The black arrows indicate connective tissue, the red arrows indicate blood vessels and the blue arrows indicate SF and PET fibers [7].

The possibility of combining nanofiber-based processing with textile technologies was recently explored for producing SF-based scaffolds for bone TE applications [97, 98]. These novel structures were fabricated by conjugated electrospinning of aqueous TSF solution combined with synthetic polylactic acid (PLA) to form nanofiber yarns. Woven scaffolds were further processed into multilayered fabrics (**Figure 7**).

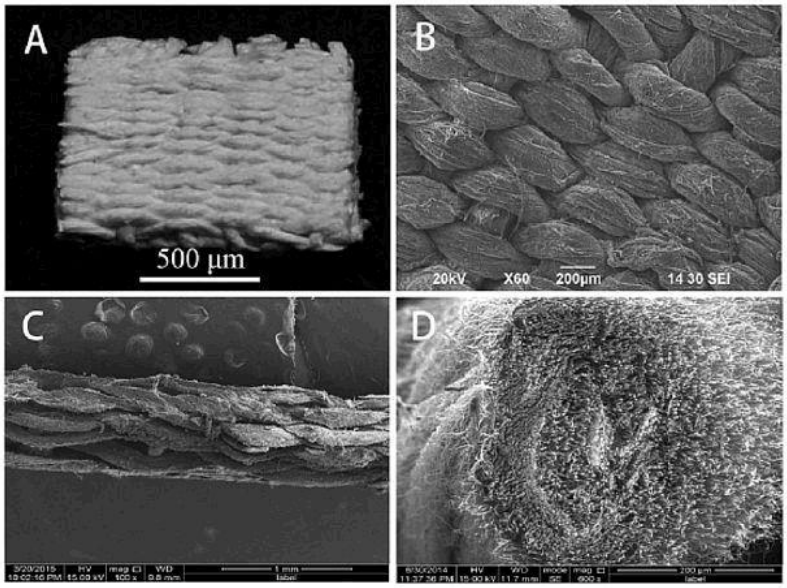


Figure 7. (A) Photograph of multilayer woven fabrics fabricated from electrospun TSF/PLA, and SEM images of the (B) surface and (C) cross-section for the scaffold, and (D) cross-section of the yarns in the scaffold. Reprinted from [97] with permission.

The authors observed that woven structures presented superior mechanical properties as compared to the electrospun non-woven nanofibers [97, 98]. Moreover, the presence of TSF showed to accelerate the growth of HA at the scaffolds immersed in simulated body fluid (SBF), and improved the compressive mechanical properties of the mineralized structures [98]. From biological evaluation, both studies showed that multilayered nanofiber-based structures with a mineralized matrix improved mesenchymal stem cells (MSCs) adhesion, proliferation and osteogenic differentiation. These woven scaffolds also promoted biomineralization after culturing, demonstrated by ALP activity and mineral deposition. The *in vivo* study revealed that scaffolds enhanced new bone formation in damaged femoral condyle of rabbits [97]. Based on the superior structural properties, excellent mechanical behavior and osteoinductivity, the authors considered that this can be a promising strategy for producing versatile and highly reproducible scaffolds for bone TE applications.

In **Table 2** are compiled the most recent studies involving SF-based biotextiles as scaffolds for bone TE, summarized in terms of processing technology and important outcomes.

Table 2. Technologies used for producing the most recent SF-based biotextiles as scaffolds for bone TE applications.

Technology	Materials	Cells/Growth factors	Outcomes	Year	Ref.
Electrospinning	TSF/HA	Osteoblast-like MG-63 cells	Nanocomposite fibers improved the mechanical properties of the scaffolds, supported MG-63 cells adhesion, proliferation and enhanced biomineralization	2016	[78]
	SF/HA	BMSCs/BMP-2	Nano-HA and BMP-2 incorporated in nanofibrous SF improved the mechanical properties of the scaffolds and stimulated BMSCs osteogenic differentiation	2017	[76]
	SF/CMC/nBG	Human MSCs	Nano-BG incorporated in nanofibrous SF/CMC improved the bioactivity and physico-chemical properties of the scaffolds, supporting hMSCs osteogenic differentiation and biomineralization	2018	[85]
	SF/GO	BMSCs/BMP-2	Functionalized GO by BMP-2 polypeptide improved the biocompatibility of eletrospun SF scaffolds, promoting BMSCs osteogenic differentiation and rat calvarial bone defects repair	2019	[82]
	SF/PCL/MgO	MC3T3-E1 cells	Controlled release of nano-MgO incorporated in SF/PCL-blend scaffolds, improving MC3T3-E1 cells osteogenic response and bone regeneration in rat calvarial defects	2020	[25]
Non-Woven	SF/HA	MG-63 cells and hBMSCs	Addition of non-woven SF fibers and HA nanocrystals improved the compressive modulus of porous SF sponges, and enhanced cellular viability and osteogenic profile of MG63 and hBMSCs cells	2016	[93]
Woven	SF/Ca-def HA	-	Ca-def HA crystallization on non-woven SF nets and woven SF fabrics improved the mechanical properties and signified the high surface area of the fabrics	2020	[94]

	TSF/PLA	Mouse MSCs	Electrospun TSF/PLA-blend nanofibers used for weaving multilayered woven fabrics positively influenced the mechanical properties of the scaffolds and supported mMSCs osteogenic differentiation, biomineralization and new bone formation in damaged femoral condyle of rabbits	2016	[97]
		MSCs	Mineralization of multilayered TSF/PLA woven fabrics by immersion in SBF improved the mechanical properties, bioactivity and MSCs osteogenic differentiation on the composite scaffolds, accelerated by the presence of TSF	2018	[98]
Weft-knitted	SF/ NaOH, UV/O_3, Plasma/AAc, Plasma/VPA, Plasma/VSA	L929 fibroblast cells	Surface functionalization of SF fabrics by NaOH, UV/O_3, Plasma/AAc, Plasma/VPA, or Plasma/VSA impacted the mechanical properties of the scaffolds, showing adequate biological support for L929 cell adhesion and proliferation	2016	[23]
	SF/PET	Human ASCs	Addition of a monofilament of PET spacing two external layers of weft-knitted SF increased the three-dimensionality of the fabrics, enhancing hASCs osteogenic differentiation, biomineralization and *in vivo* angiogenesis	2017	[7]

CMC: carboxymethyl cellulose; nBG: nano-bioglass

5. Concluding Remarks

The design of an ideal scaffold for bone tissue regeneration and repair remains a challenge, and most of the existing scaffolding strategies do not satisfy all the requirements of biocompatibility, biodegradability, porosity, interconnectivity, and surface properties. From the overviewed reports, it is evident that major advances were made in the nanofiber, textile, and polymer sciences that contributed for the development of novel scaffold materials for bone TE applications. Through these technologies, different fiber sizes and cross-sections can be reached, allowing us to manipulate the fabric structure according to the desired application. The mechanical strength, microstructure and 3D architecture can also be tuned in order to possibly modulating cell recognition, proliferation and differentiation. Naturally-derived polymers have been showing advantages of biocompatibility and biodegradability that are essential for bone TE strategies. However, their application in bone TE field can be limited by their lack of mechanical strength. SF has shown remarkable potential for bone TE applications presenting superior structural and mechanical properties for the design of more complex 3D structures. Furthermore, the possibility of using SF for textile processing in its native state (directly obtained from cocoons) or from regenerated aqueous SF solutions, brings a series of possibilities for creating highly reproducible, biocompatible and mechanically superior scaffolds for bone regeneration applications.

Acknowledgments
The authors thank to the project FROnTHERA (NORTE-01-0145-FEDER-000023), supported by Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF). The financial support from the Portuguese Foundation for Science and Technology to Fun4TE project (PTDC/EMD-EMD/31367/2017), for the Junior Researcher contract (POCI-01-0145-FEDER-031367), and for the FCT distinction attributed to J. M. Oliveira under the Investigador FCT program (IF/01285/2015) are also greatly acknowledged.

References

1. T. Ghassemi, A. Shahroodi, M.H. Ebrahimzadeh, A. Mousavian, J. Movaffagh, A. Moradi, Current concepts in scaffolding for bone tissue engineering. Archives of bone and joint surgery. **6**(2): 90 (2018).
2. V.P. Ribeiro, A. da Silva Morais, F.R. Maia, R.F. Canadas, J.B. Costa, A.L. Oliveira, J.M. Oliveira, R.L. Reis, Combinatory approach for developing silk fibroin scaffolds for cartilage regeneration. Acta Biomater. **72**: 167-181 (2018).
3. C.R. Carvalho, J.B. Costa, A. da Silva Morais, R. López-Cebral, J. Silva-Correia, R.L. Reis, J.M. Oliveira, Tunable Enzymatically Cross-Linked Silk Fibroin Tubular Conduits for Guided Tissue Regeneration. Advanced Healthcare Materials. **7**(17): 1800186 (2018).
4. J. Hahn, G. Schulze-Tanzil, M. Schröpfer, M. Meyer, C. Gögele, M. Hoyer, A. Spickenheuer, G. Heinrich, A. Breier, Viscoelastic behavior of embroidered scaffolds for ACL tissue engineering made of PLA and P (LA-CL) after in vitro degradation. International journal of molecular sciences. **20**(18): 4655 (2019).
5. F.S. Frueh, M.D. Menger, N. Lindenblatt, P. Giovanoli, M.W. Laschke, Current and emerging vascularization strategies in skin tissue engineering. Critical reviews in biotechnology. (2016).
6. H. Qu, H. Fu, Z. Han, Y. Sun, Biomaterials for bone tissue engineering scaffolds: a review. RSC advances. **9**(45): 26252-26262 (2019).
7. V.P. Ribeiro, J. Silva-Correia, A.I. Nascimento, A. da Silva Morais, A.P. Marques, A.S. Ribeiro, C.J. Silva, G. Bonifácio, R.A. Sousa, J.M. Oliveira, Silk-based anisotropical 3D biotextiles for bone regeneration. Biomaterials. **123**: 92-106 (2017). doi:10.1016/j.biomaterials.2017.01.027.
8. V.P. Ribeiro, S. Pina, J.o.B. Costa, I.F. Cengiz, L. García-Fernández, M.d.M. Fernández-Gutiérrez, O.C. Paiva, A.L. Oliveira, J. San-Román, J.M. Oliveira, Enzymatically cross-linked silk fibroin-based hierarchical scaffolds for osteochondral regeneration. ACS applied materials & interfaces. **11**(4): 3781-3799 (2019). doi:10.1021/acsami.8b21259.
9. D.P. Bhattarai, L.E. Aguilar, C.H. Park, C.S. Kim, A review on properties of natural and synthetic based electrospun fibrous materials for bone tissue engineering. Membranes. **8**(3): 62 (2018).
10. X. Wang, T. Lou, W. Zhao, G. Song, C. Li, G. Cui, The effect of fiber size and pore size on cell proliferation and infiltration in PLLA scaffolds on bone tissue engineering. Journal of biomaterials applications. **30**(10): 1545-1551 (2016).
11. M.W. King, Designing fabrics for blood vessel replacement. Canadian Textile Journal. **108**(4): 24-30 (1991).
12. S. Tuin, B. Pourdeyhimi, E. Loboa, Creating tissues from textiles: scalable nonwoven manufacturing techniques for fabrication of tissue engineering scaffolds. Biomedical Materials. **11**(1): 015017 (2016).
13. S. Salehi, M. Kharaziha, N. Masoumi, A. Fallahi, A. Tamayol, Medical textiles as substrates for tissue engineering. Textile Finishing: Recent Developments and Future Trends. 363-421 (2017).
14. L.R. Almeida, A.R. Martins, E.M. Fernandes, M.B. Oliveira, J.F. Mano, V.M. Correlo, I. Pashkuleva, A.P. Marques, A.S. Ribeiro, N.F. Duraes, C.J. Silva, G. Bonifacio, R.A. Sousa, A.L. Oliveira, R.L. Reis, New biotextiles for tissue engineering: Development, characterization and in vitro cellular viability. Acta Biomater. **9**(11): 9241-9241 (2013). doi:10.1016/j.actbio.2013.05.019.
15. A. Walther, B. Hoyer, A. Springer, B. Mrozik, T. Hanke, C. Cherif, W. Pompe, M. Gelinsky, Novel textile scaffolds generated by flock technology for tissue engineering of bone and cartilage. Materials. **5**(3): 540-557 (2012).
16. J.G. Barber, A.M. Handorf, T.J. Allee, W.-J. Li, Braided nanofibrous scaffold for tendon and ligament tissue engineering. Tissue Eng Pt A. **19**(11-12): 1265-1274 (2013).

17. J. Gilmore, T. Burg, R.E. Groff, K.J. Burg, Design and optimization of a novel bio-loom to weave melt-spun absorbable polymers for bone tissue engineering. Journal of Biomedical Materials Research Part B: Applied Biomaterials. **105**(6): 1342-1351 (2017).
18. A. Davies, J. Williams, The use of spacer fabrics for absorbent medical applications. J Fiber Bioeng Inform. **1**(4): 321-30 (2009). doi:10.3993/jfbi03200910.
19. F. Donnaloja, E. Jacchetti, M. Soncini, M.T. Raimondi, Natural and Synthetic Polymers for Bone Scaffolds Optimization. Polymers. **12**(4): 905 (2020).
20. S.H. Rao, B. Harini, R.P.K. Shadamarshan, K. Balagangadharan, N. Selvamurugan, Natural and synthetic polymers/bioceramics/bioactive compounds-mediated cell signalling in bone tissue engineering. International journal of biological macromolecules. **110**: 88-96 (2018).
21. T.P. Nguyen, Q.V. Nguyen, V.-H. Nguyen, T.-H. Le, V.Q.N. Huynh, D.-V.N. Vo, Q.T. Trinh, S.Y. Kim, Q.V. Le, Silk fibroin-based biomaterials for biomedical applications: A review. Polymers. **11**(12): 1933 (2019).
22. T. Katashima, A.D. Malay, K. Numata, Chemical modification and biosynthesis of silk-like polymers. Current Opinion in Chemical Engineering. **24**: 61-68 (2019).
23. V.P. Ribeiro, L.R. Almeida, A.R. Martins, I. Pashkuleva, A.P. Marques, A.S. Ribeiro, C.J. Silva, G. Bonifacio, R.A. Sousa, R.L. Reis, Influence of different surface modification treatments on silk biotextiles for tissue engineering applications. Journal of Biomedical Materials Research Part B: Applied Biomaterials. **104**(3): 496-507 (2016). doi:10.1002/jbm.b.33400.
24. A. Wubneh, E.K. Tsekoura, C. Ayranci, H. Uludağ, Current state of fabrication technologies and materials for bone tissue engineering. Acta Biomater. **80**: 1-30 (2018).
25. X. Xing, G. Cheng, C. Yin, X. Cheng, Y. Cheng, Y. Ni, X. Zhou, H. Deng, Z. Li, Magnesium-Containing Silk Fibroin/Polycaprolactone Electrospun Nanofibrous Scaffolds for Accelerating Bone Regeneration. Arabian Journal of Chemistry. **13**(5): 5526-5538 (2020). doi:10.1016/j.arabjc.2020.03.031.
26. B. Ten Harkel, T. Schoenmaker, D.I. Picavet, N.L. Davison, T.J. De Vries, V. Everts, The foreign body giant cell cannot resorb bone, but dissolves hydroxyapatite like osteoclasts. PloS one. **10**(10): e0139564 (2015). doi:10.1371/journal.pone.0139564.
27. J. Di Martino, E. Henriet, Z. Ezzoukhry, J.G. Goetz, V. Moreau, F. Saltel, The microenvironment controls invadosome plasticity. J Cell Sci. **129**(9): 1759-68 (2016). doi:10.1242/jcs.182329.
28. R. Florencio-Silva, G.R.d.S. Sasso, E. Sasso-Cerri, M.J. Simões, P.S. Cerri, Biology of bone tissue: structure, function, and factors that influence bone cells. BioMed research international. **2015** (2015). doi:10.1155/2015/421746.
29. N.A. Sims, C. Vrahnas, Regulation of cortical and trabecular bone mass by communication between osteoblasts, osteocytes and osteoclasts. Archives of biochemistry and biophysics. **561**: 22-28 (2014). doi:10.1016/j.abb.2014.05.015.
30. G.G. Walmsley, R.C. Ransom, E.R. Zielins, T. Leavitt, J.S. Flacco, M.S. Hu, A.S. Lee, M.T. Longaker, D.C. Wan, Stem cells in bone regeneration. Stem Cell Reviews and Reports. **12**(5): 524-529 (2016).
31. Z. Qi, W. Liu, J. Lu, The mechanisms underlying the beneficial effects of exercise on bone remodeling: roles of bone-derived cytokines and microRNAs. Progress in Biophysics and Molecular biology. **122**(2): 131-139 (2016).
32. G. Carmeliet, V. Dermauw, R. Bouillon, Vitamin D signaling in calcium and bone homeostasis: a delicate balance. Best Practice & Research Clinical Endocrinology & Metabolism. **29**(4): 621-631 (2015). doi:10.1016/j.beem.2015.06.001.
33. B. Kundu, N.E. Kurland, S. Bano, C. Patra, F.B. Engel, V.K. Yadavalli, S.C. Kundu, Silk proteins for biomedical applications: bioengineering perspectives. Prog Polym Sci. **39**(2): 251-267 (2014). doi:10.1016/j.progpolymsci.2013.09.002.

34. S. Salehi, K. Koeck, T. Scheibel, Spider silk for tissue engineering applications. Molecules. **25**(3): 737 (2020).
35. J. Melke, S. Midha, S. Ghosh, K. Ito, S. Hofmann, Silk fibroin as biomaterial for bone tissue engineering. Acta Biomater. **31**: 1-16 (2016). doi:10.1016/j.actbio.2015.09.005.
36. P. Bhattacharjee, B. Kundu, D. Naskar, H.-W. Kim, T.K. Maiti, D. Bhattacharya, S.C. Kundu, Silk scaffolds in bone tissue engineering: An overview. Acta Biomater. **63**: 1-17 (2017). doi:10.1016/j.actbio.2017.09.027.
37. Z. Karahaliloglu, E. Kilicay, E.B. Denkbas, Antibacterial chitosan/silk sericin 3D porous scaffolds as a wound dressing material. Artificial cells, nanomedicine, and biotechnology. **45**(6): 1172-1185 (2017). doi:10.1080/21691401.2016.1203796.
38. T. Siritientong, A. Angspatt, J. Ratanavaraporn, P. Aramwit, Clinical potential of a silk sericin-releasing bioactive wound dressing for the treatment of split-thickness skin graft donor sites. Pharmaceutical research. **31**(1): 104-116 (2014). doi:10.1007/s11095-013-1136-y.
39. J.P. Kumar, B.B. Mandal, Silk sericin induced pro-oxidative stress leads to apoptosis in human cancer cells. Food and Chemical Toxicology. **123**: 275-287 (2019).
40. M.C. Arango, Y. Montoya, M.S. Peresin, J. Bustamante, C. Álvarez-López, Silk sericin as a biomaterial for tissue engineering: a review. International Journal of Polymeric Materials and Polymeric Biomaterials. 1-15 (2020).
41. M. McGill, J.M. Coburn, B.P. Partlow, X. Mu, D.L. Kaplan, Molecular and macro-scale analysis of enzyme-crosslinked silk hydrogels for rational biomaterial design. Acta Biomater. **63**: 76-84 (2017). doi:10.1016/j.actbio.2017.09.020.
42. L.-D. Koh, Y. Cheng, C.-P. Teng, Y.-W. Khin, X.-J. Loh, S.-Y. Tee, M. Low, E. Ye, H.-D. Yu, Y.-W. Zhang, Structures, mechanical properties and applications of silk fibroin materials. Prog Polym Sci. **46**: 86-110 (2015). doi:10.1016/j.progpolymsci.2015.02.001.
43. B. Kundu, R. Rajkhowa, S.C. Kundu, X.G. Wang, Silk fibroin biomaterials for tissue regenerations. Adv Drug Deliver Rev. **65**(4): 457-470 (2013). doi:10.1016/j.addr.2012.09.043.
44. S.S. Silva, E.G. Popa, M.E. Gomes, M.B. Oliveira, S. Nayak, B. Subia, J.F. Mano, S.C. Kundu, R.L. Reis, Silk hydrogels from non-mulberry and mulberry silkworm cocoons processed with ionic liquids. Acta Biomater. **9**(11): 8972-8982 (2013). doi:10.1016/j.actbio.2013.06.044.
45. V.P. Ribeiro, J. Silva-Correia, C. Gonçalves, S. Pina, H. Radhouani, T. Montonen, J. Hyttinen, A. Roy, A.L. Oliveira, R.L. Reis, Rapidly responsive silk fibroin hydrogels as an artificial matrix for the programmed tumor cells death. PloS one. **13**(4): e0194441 (2018). doi:10.1371/journal.pone.0194441.
46. L.-P. Yan, J. Silva-Correia, V.P. Ribeiro, V. Miranda-Gonçalves, C. Correia, A. da Silva Morais, R.A. Sousa, R.M. Reis, A.L. Oliveira, J.M. Oliveira, Tumor growth suppression induced by biomimetic silk fibroin hydrogels. Scientific reports. **6**: 31037 (2016). doi:10.1038/srep31037.
47. J.-H. Lee, H.-W. Kim, Emerging properties of hydrogels in tissue engineering. Journal of tissue engineering. **9**: 2041731418768285 (2018). doi:10.1177/2041731418768285.
48. M.G. Manda, L.P. da Silva, M.T. Cerqueira, D.R. Pereira, M.B. Oliveira, J.F. Mano, A.P. Marques, J.M. Oliveira, V.M. Correlo, R.L. Reis, Gellan gum-hydroxyapatite composite spongy-like hydrogels for bone tissue engineering. J Biomed Mater Res A. **106**(2): 479-490 (2018). doi:10.1002/jbm.a.36248.
49. Y.P. Singh, N. Bhardwaj, B.B. Mandal, Potential of agarose/silk fibroin blended hydrogel for in vitro cartilage tissue engineering. ACS applied materials & interfaces. **8**(33): 21236-21249 (2016).
50. A. Zuluaga-Vélez, D.F. Cómbita-Merchán, R. Buitrago-Sierra, J.F. Santa, E. Aguilar-Fernández, J.C. Sepúlveda-Arias, Silk fibroin hydrogels from the Colombian silkworm Bombyx mori L: Evaluation of physicochemical properties. PloS one. **14**(3): e0213303 (2019).

51. A. Matsumoto, J. Chen, A.L. Collette, U.-J. Kim, G.H. Altman, P. Cebe, D.L. Kaplan, Mechanisms of silk fibroin sol− gel transitions. The Journal of Physical Chemistry B. **110**(43): 21630-21638 (2006). doi:10.1021/jp056350v.
52. S.-D. Wang, K.-Q. Zhang, Electrogelation and rapid prototyping of Bombyx mori silk fibroin. Materials Letters. **169**: 5-9 (2016). doi:10.1016/j.matlet.2016.01.079.
53. V.P. Ribeiro, S.C.A. Pina, R.F. Canadas, A. Morais, C. Vilela, S.C.A. Vieira, I.F. Cengiz, R.L. Reis, J.M. Oliveira, In vivo performance of hierarchical HRP-crosslinked silk fibroin/β-TCP scaffolds for osteochondral tissue regeneration. Regenerative Medicine Frontiers. **1**: e190007 (2019). doi:10.20900/rmf20190007.
54. V.P. Ribeiro, S.C.A. Pina, S. Gheduzzi, A.C.P. Araújo, R.L. Reis, J.M. Oliveira, Hierarchical HRP-crosslinked silk fibroin/ZnSr-TCP scaffolds for osteochondral tissue regeneration: Assessment of the mechanical and antibacterial properties. Frontiers in Materials. **7**: 49 (2020). doi:10.3389/fmats.2020.00049.
55. L.-P. Yan, J. Silva-Correia, M.B. Oliveira, C. Vilela, H. Pereira, R.A. Sousa, J.F. Mano, A.L. Oliveira, J.M. Oliveira, R.L. Reis, Bilayered silk/silk-nanoCaP scaffolds for osteochondral tissue engineering: in vitro and in vivo assessment of biological performance. Acta Biomater. **12**: 227-241 (2015). doi: 10.1016/j.actbio.2014.10.021.
56. L.-P. Yan, J. Silva-Correia, C. Correia, S.G. Caridade, E.M. Fernandes, R.A. Sousa, J.F. Mano, J.M. Oliveira, A.L. Oliveira, R.L. Reis, Bioactive macro/micro porous silk fibroin/nano-sized calcium phosphate scaffolds with potential for bone-tissue-engineering applications. Nanomedicine. **8**(3): 359-378 (2013). doi:10.2217/nnm.12.118.
57. L.-P. Yan, A.J. Salgado, J.M. Oliveira, A.L. Oliveira, R.L. Reis, De novo bone formation on macro/microporous silk and silk/nano-sized calcium phosphate scaffolds. J Bioact Compat Pol. **28**(5): 439-452 (2013). doi:10.1177/0883911513503538.
58. L. Wei, S. Wu, M. Kuss, X. Jiang, R. Sun, P. Reid, X. Qin, B. Duan, 3D printing of silk fibroin-based hybrid scaffold treated with platelet rich plasma for bone tissue engineering. Bioactive materials. **4**: 256-260 (2019). doi:10.1016/j.bioactmat.2019.09.001.
59. S. Chawla, S. Midha, A. Sharma, S. Ghosh, Silk-based bioinks for 3D bioprinting. Advanced healthcare materials. **7**(8): 1701204 (2018). doi:10.1002/adhm.201701204.
60. J.B. Costa, J. Silva-Correia, J.M. Oliveira, R.L. Reis, Fast setting silk fibroin bioink for bioprinting of patient-specific memory-shape implants. Advanced healthcare materials. **6**(22): 1701021 (2017). doi:10.1002/adhm.201701021.
61. J.B. Costa, J. Silva-Correia, V.P. Ribeiro, A. da Silva Morais, J.M. Oliveira, R.L. Reis, Engineering patient-specific bioprinted constructs for treatment of degenerated intervertebral disc. Materials Today Communications. **19**: 506-512 (2019). doi:10.1016/j.mtcomm.2018.01.011.
62. S. Pina, R. Canadas, G. Jiménez, M. Perán, J. Marchal, R. Reis, J. Oliveira, Biofunctional ionic-doped calcium phosphates: silk fibroin composites for bone tissue engineering scaffolding. Cells Tissues Organs. **204**(3-4): 150-163 (2017). doi:10.1159/000469703.
63. M. Ribeiro, M.A. de Moraes, M.M. Beppu, M.P. Garcia, M.H. Fernandes, F.J. Monteiro, M.P. Ferraz, Development of silk fibroin/nanohydroxyapatite composite hydrogels for bone tissue engineering. European polymer journal. **67**: 66-77 (2015). doi:10.1016/j.eurpolymj.2015.03.056.
64. M. Farokhi, F. Mottaghitalab, S. Samani, M.A. Shokrgozar, S.C. Kundu, R.L. Reis, Y. Fatahi, D.L. Kaplan, Silk fibroin/hydroxyapatite composites for bone tissue engineering. Biotechnology advances. **36**(1): 68-91 (2018). doi:10.1016/j.biotechadv.2017.10.001.
65. W.J. Li, C.T. Laurencin, E.J. Caterson, R.S. Tuan, F.K. Ko, Electrospun nanofibrous structure: a novel scaffold for tissue engineering. J Biomed Mater Res. **60**(4): 613-621 (2002). doi:10.1002/jbm.10167.

66. A. Martins, M.L. Alves da Silva, S. Faria, A.P. Marques, R.L. Reis, N.M. Neves, The influence of patterned nanofiber meshes on human mesenchymal stem cell osteogenesis. Macromol Biosci. **11**(7): 978-987 (2011). doi:10.1002/mabi.201100012.
67. R.F. Pereira, M.M. Silva, V. de Zea Bermudez, Bombyx mori silk fibers: An outstanding family of materials. Macromolecular Materials and Engineering. **300**(12): 1171-1198 (2015). doi:10.1002/mame.201400276.
68. B. Kundu, R. Rajkhowa, S.C. Kundu, X. Wang, Silk fibroin biomaterials for tissue regenerations. Adv Drug Deliver Rev. **65**(4): 457-470 (2013). doi:10.1016/j.addr.2012.09.043.
69. C.P. Barnes, S.A. Sell, E.D. Boland, D.G. Simpson, G.L. Bowlin, Nanofiber technology: designing the next generation of tissue engineering scaffolds. Adv Drug Deliver Rev. **59**(14): 1413-1433 (2007). doi:10.1016/j.addr.2007.04.022.
70. S. Khorshidi, A. Solouk, H. Mirzadeh, S. Mazinani, J.M. Lagaron, S. Sharifi, S. Ramakrishna, A review of key challenges of electrospun scaffolds for tissue-engineering applications. J Tissue Eng Regen M. **10**(9): 715-738 (2016). doi:10.1002/term.1978.
71. J. Xue, T. Wu, Y. Dai, Y. Xia, Electrospinning and electrospun nanofibers: methods, materials, and applications. Chemical reviews. **119**(8): 5298-5415 (2019).
72. K.-H. Kim, L. Jeong, H.-N. Park, S.-Y. Shin, W.-H. Park, S.-C. Lee, T.-I. Kim, Y.-J. Park, Y.-J. Seol, Y.-M. Lee, Biological efficacy of silk fibroin nanofiber membranes for guided bone regeneration. J Biotechnol. **120**(3): 327-339 (2005). doi:10.1016/j.jbiotec.2005.06.033.
73. H.-J. Jin, J. Chen, V. Karageorgiou, G.H. Altman, D.L. Kaplan, Human bone marrow stromal cell responses on electrospun silk fibroin mats. Biomaterials. **25**(6): 1039-1047 (2004). doi:10.1016/s0142-9612(03)00609-4.
74. C. Meechaisue, P. Wutticharoenmongkol, R. Waraput, T. Huangjing, N. Ketbumrung, P. Pavasant, P. Supaphol, Preparation of electrospun silk fibroin fiber mats as bone scaffolds: a preliminary study. Biomedical Materials. **2**(3): 181 (2007). doi:10.1088/1748-6041/2/3/003.
75. H. Kim, L. Che, Y. Ha, W. Ryu, Mechanically-reinforced electrospun composite silk fibroin nanofibers containing hydroxyapatite nanoparticles. Materials Science and Engineering: C. **40**: 324-335 (2014). doi:10.1016/j.msec.2014.04.012.
76. B. Niu, B. Li, Y. Gu, X. Shen, Y. Liu, L. Chen, In vitro evaluation of electrospun silk fibroin/nano-hydroxyapatite/BMP-2 scaffolds for bone regeneration. Journal of Biomaterials Science, Polymer Edition. **28**(3): 257-270 (2017). doi:10.1080/09205063.2016.1262163.
77. S.Y. Yang, T.H. Hwang, L. Che, J.S. Oh, Y. Ha, W. Ryu, Membrane-reinforced three-dimensional electrospun silk fibroin scaffolds for bone tissue engineering. Biomedical Materials. **10**(3): 035011 (2015). doi:10.1088/1748-6041/10/3/035011.
78. W. Shao, J. He, F. Sang, B. Ding, L. Chen, S. Cui, K. Li, Q. Han, W. Tan, Coaxial electrospun aligned tussah silk fibroin nanostructured fiber scaffolds embedded with hydroxyapatite–tussah silk fibroin nanoparticles for bone tissue engineering. Materials Science and Engineering: C. **58**: 342-351 (2016). doi:10.1016/j.msec.2015.08.046.
79. Z. Hadisi, J. Nourmohammadi, J. Mohammadi, Composite of porous starch-silk fibroin nanofiber-calcium phosphate for bone regeneration. Ceramics International. **41**(9): 10745-10754 (2015). doi:10.1016/j.ceramint.2015.05.010.
80. E.I. Paşcu, J. Stokes, G.B. McGuinness, Electrospun composites of PHBV, silk fibroin and nano-hydroxyapatite for bone tissue engineering. Materials Science and Engineering: C. **33**(8): 4905-4916 (2013). doi:10.1016/j.msec.2013.08.012.
81. C. Li, C. Vepari, H.-J. Jin, H.J. Kim, D.L. Kaplan, Electrospun silk-BMP-2 scaffolds for bone tissue engineering. Biomaterials. **27**(16): 3115-3124 (2006). doi:10.1016/j.biomaterials.2006.01.022.
82. J. Wu, A. Zheng, Y. Liu, D. Jiao, D. Zeng, X. Wang, L. Cao, X. Jiang, Enhanced bone regeneration of the silk fibroin electrospun scaffolds through the modification of the graphene oxide

functionalized by BMP-2 peptide. International journal of nanomedicine. **14**: 733 (2019). doi:10.2147/IJN.S187664.
83. W. Shao, J. He, F. Sang, Q. Wang, L. Chen, S. Cui, B. Ding, Enhanced bone formation in electrospun poly (l-lactic-co-glycolic acid)–tussah silk fibroin ultrafine nanofiber scaffolds incorporated with graphene oxide. Materials Science and Engineering: C. **62**: 823-834 (2016). doi:10.1016/j.msec.2016.01.078.
84. G.-J. Lai, K. Shalumon, S.-H. Chen, J.-P. Chen, Composite chitosan/silk fibroin nanofibers for modulation of osteogenic differentiation and proliferation of human mesenchymal stem cells. Carbohydrate polymers. **111**: 288-297 (2014). doi:10.1016/j.carbpol.2014.04.094.
85. B. Singh, K. Pramanik, Generation of bioactive nano-composite scaffold of nanobioglass/silk fibroin/carboxymethyl cellulose for bone tissue engineering. Journal of Biomaterials Science, Polymer Edition. **29**(16): 2011-2034 (2018). doi:10.1080/09205063.2018.1523525.
86. B.M. Roux, M.H. Cheng, E.M. Brey, Engineering clinically relevant volumes of vascularized bone. J Cell Mol Med. **19**(5): 903-914 (2015). doi:10.1111/jcmm.12569.
87. M. Kun, C. Chan, S. Ramakrishna, in *Advanced textiles for wound care*, ed. by A. Kulkarni, K. Vadodaria (Woodhead Publishing, 2019), p. 329-362.
88. M. Akbari, A. Tamayol, S. Bagherifard, L. Serex, P. Mostafalu, N. Faramarzi, M.H. Mohammadi, A. Khademhosseini, Textile technologies and tissue engineering: a path toward organ weaving. Advanced healthcare materials. **5**(7): 751-766 (2016). doi:10.1002/adhm.201500517.
89. X. Zhang, P. Ma, Application of knitting structure textiles in medical areas. AUTEX Research Journal. **18**(2): 181-191 (2018). doi:10.1515/aut-2017-0019.
90. X. Wang, C. Han, X. Hu, H. Sun, C. You, C. Gao, Y. Haiyang, Applications of knitted mesh fabrication techniques to scaffolds for tissue engineering and regenerative medicine. Journal of the mechanical behavior of biomedical materials. **4**(7): 922-932 (2011). doi:10.1016/j.jmbbm.2011.04.009.
91. S. Rajendran, S.C. Anand, in *Woven Textiles: Principles, Technologies and Applications*, ed. by K.L. Gandhi (Elsevier, 2020), p. 441-470.
92. G. Li, Y. Li, G. Chen, J. He, Y. Han, X. Wang, D.L. Kaplan, Silk-based biomaterials in biomedical textiles and fiber-based implants. Advanced healthcare materials. **4**(8): 1134-1151 (2015). doi:10.1002/adhm.201500002.
93. P. Gupta, M. Adhikary, M. Kumar, N. Bhardwaj, B.B. Mandal, Biomimetic, osteoconductive non-mulberry silk fiber reinforced tricomposite scaffolds for bone tissue engineering. ACS applied materials & interfaces. **8**(45): 30797-30810 (2016). doi:10.1021/acsami.6b11366.
94. P. Pankaew, P. White, Crystallization of Calcium Deficient Hydroxyapatite Nanocrystals on Woven Silk Fibroin Fabric via Precipitation Process. Journal of nanoscience and nanotechnology. **20**(1): 81-86 (2020). doi: 10.1166/jnn.2020.17285.
95. N. Karp, M. Choi, D.A. Kulber, S. Downey, G. Duda, G.M. Kind, M.L. Jewell, D.K. Murphy, M.R. Lehfeldt, N. Fine, SERI surgical scaffold in 2-stage breast reconstruction: 2-year data from a prospective, multicenter trial. Plastic and Reconstructive Surgery Global Open. **5**(5) (2017). doi:10.1097/GOX.0000000000001327.
96. B. Schäfer, C. Emonts, N. Glimpel, T. Ruhl, A.S. Obrecht, S. Jockenhoevel, T. Gries, J.P. Beier, A. Blaeser, Warp-Knitted Spacer Fabrics: A Versatile Platform to Generate Fiber-Reinforced Hydrogels for 3D Tissue Engineering. Materials. **13**(16): 3518 (2020).
97. W. Shao, J. He, Q. Han, F. Sang, Q. Wang, L. Chen, S. Cui, B. Ding, A biomimetic multilayer nanofiber fabric fabricated by electrospinning and textile technology from polylactic acid and Tussah silk fibroin as a scaffold for bone tissue engineering. Materials Science and Engineering: C. **67**: 599-610 (2016). doi:10.1016/j.msec.2016.05.081.

98. Y. Gao, W. Shao, W. Qian, J. He, Y. Zhou, K. Qi, L. Wang, S. Cui, R. Wang, Biomineralized poly (l-lactic-co-glycolic acid)-tussah silk fibroin nanofiber fabric with hierarchical architecture as a scaffold for bone tissue engineering. Materials Science and Engineering: C. **84**: 195-207 (2018). doi: 10.1016/j.msec.2017.11.047.