

NANOFIBROUS SCAFFOLDS OF ELECTROSPUN SILK FIBROIN/POLY(VINYL ALCOHOL) BLENDS FOR TISSUE ENGINEERING

Apinya Raksa^{1,4}, Piya-on Numpaisal^{2,4}, Rapee Utke¹, Chaiwat Ruksakulpiwat^{3,4}, and Yupaporn Ruksakulpiwat^{3,4,*}

Received: March 13, 2021; Revised: June 14, 2021; Accepted: June 22, 2021

Abstract

Electrospinning is a beneficial polymer fabrication process. It is applicable for electrospun nanofibrous scaffold which can mimic nano-scale fibrous component in the extracellular matrix of many tissues. Silk fibroin (SF) is a fibrous protein, mainly produced by silkworms. SF is composed of beta- sheet crystalline and amorphous matrix. It possesses excellent biocompatibility, biodegradability, and cell induction property. Therefore, SF is a promising resource for biomedical materials. Polyvinyl alcohol (PVA) is a semicrystalline hydrophilic polymer. According to their mechanical properties and non-toxicity, PVA is good candidate to use in a high scaffold that required high mechanical strength. In our research, the SF/PVA composite scaffold was fabricated using electrospinning technique, aimed to developed biomimetic scaffold of the meniscus, a fibrocartilage in the knee joint, which provided good biological and mechanical properties. This study focused on the effect of PVA contents in SF/ PVA nanofibrous scaffold. The processability, morphology and hydrophilicity of SF/PVA nanofibers were determined. The results showed a decrease in water contact angle with increasing PVA content in the SF/PVA nanofibers. The results also suggest that a high PVA content in the SF/PVA nanofibers can enhance the cell attachment capability of the scaffold.

Keywords: Nanofibrous, electrospinning, scaffold, tissue engineering, meniscus

Introduction

Meniscus is the fibrocartilage tissue in the knee joint. There are the medial and the lateral meniscus in the knee joint. They play an important role in distributing mechanical stress, enhances the stability of the knee joint and protects articular cartilage

(Chen *et al.*, 2018). Meniscus has complex extracellular matrix, the major component is collagen fibers, which align in different directions. The blood supply of the meniscus is limited to the outer 1/3 that has a high potential to heal. (Abrams

¹ School of Chemistry, Institute of Science, Suranaree University of Technology, Nakhon Ratchasima, 30000, Thailand.

² School of Orthopaedics, Institute of Medicine, Suranaree University of Technology, Nakhon Ratchasima, 30000, Thailand.

³ School of Polymer Engineering, Institute of Engineering, Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand. E-mail: yupa@sut.ac.th

⁴ Research Center for Biocomposite Materials for Medical Industry and Agricultural and Food Industry, Nakhon Ratchasima 30000, Thailand.

* Corresponding author

et al., 2013). Meniscus tear is a common injury among injury in the knee joint. It can be a result from sports or degeneration. However, meniscal injury in the medial meniscus takes place more frequently than in the lateral meniscus (Baker *et al.*, 1985; Hede *et al.*, 1990; Campbell *et al.*, 2001). Irreparable injured meniscus can cause knee pain and disability. Nevertheless, many techniques have been developed for meniscus repair, such as sutures and implant fixation (Roos *et al.*, 1998; Englund *et al.*, 2010; Chang *et al.*, 2011). However, due to the drawback of surgical treatment, there is a need to eliminate this limitation. For this reason, tissue engineering has become an alternative treatment option for meniscus injury. Tissue Engineering (TE) is the creation of a new tissue to replace, restore and maintain the functions of any part of the body. TE has three major components; cells, bioactive molecules, and scaffolds. Generally, the scaffold requires these basic properties; biodegradability, biocompatibility, non-toxicity, and cell conductivity. Currently, scaffolds have been extensively used in the field of biomedical applications (Ross *et al.*, 2017). Scaffold in tissue engineering for musculoskeletal system, such as skin, nerve tissue, tendon, cartilage, and meniscus (Halili, 2011) are from various polymers. Polymers can be derived into natural polymers (i.e. collagen, chitosan, silk fibroin, alginate, albumin, starch) and synthetic polymers (i.e. poly (vinyl alcohol), polylactide, polycaprolactone, poly (lactic acid), polyethylene glycol) (Panilaitis *et al.*, 2003; Tarun *et al.*, 2011; Ross *et al.*, 2017). The advantage of natural polymers are biocompatibility, biodegradability, nontoxicity to the human body. Additionally, it is similar to extracellular matrix component of human tissues. Anyway, natural polymers have limited availability, expensive, natural variety and susceptible to cross-contamination. The advantage of synthetic polymers is they are easier to control physicochemical properties (Agarwal *et al.*, 2008; Sill *et al.*, 2008; Gunn and Zhang, 2010; Ross *et al.*, 2017) but poor cell adhesion. In order to obtain the scaffold with good biological support and mechanical properties, silk fibroin and poly(vinyl alcohol) were used to fabricate into a biocomposite electrospun nanofibers.

Silk fibers from silk cocoon (*Bombyx mori*) comprises two structural proteins: silk fibroin (72-81 wt%) and sericin (19-28 wt%). Silk fibroin (SF) is a major structural protein, contained polypeptide chains with a molecular weight in the range of 25-350 kDa (Su *et al.*, 2019). The main structure of SF consists of repetitive blocks of hydrophobic heavy chains (H-fibroin) and hydrophilic light chains (L-chain) (Nguyen *et al.*, 2019; Sakunphanitphan *et al.*, 2019). In H-fibroin, there are 45.9% of Gly, 30.3% of Ala, 12.1% of Ser, 5.3%

of tyrosine (Tyr), and 1.8% of valine (Val). H-fibroin folds and bonds together via Van der Waals forces, hydrogen bonds, and hydrophobic interactions to form anti-parallel β -sheet crystalline structures (Huang *et al.*, 2018). On the other hand, L-chain comprises of amino acid sequences that contain 14% alanine (Ala), 10% serine (Ser), 9% glycine (Gly) (Nguyen *et al.*, 2019). SF is a good candidate for bioscaffold. It has good biocompatibility and biodegradability. The limitation of SF is low mechanical strength. From this reason, SF generally needs to be blended with other to material which provided better mechanical property. PVA has been widely used in biomedical application. It possesses excellent mechanical strength, low cost and non-toxic (Kumkun *et al.*, 2019).

In our study, electrospinning was used to fabricate SF/PVA based nanofibers. Electrospinning, also known as electrostatic spinning. It is a process of polymer fiber fabrication, utilized a strong electric field generated by a high voltage power supply between a polymer containing fluid and a grounded conducting collector (Thompson *et al.*, 2007). The high voltage generates an electrically charged jet which ejects from a needle and undergoes very large stretching. During injection and at the arrival of the conducting collector, the solvent evaporates from the polymer solution, creating polymeric fiber. The creation of a scaffold from electrospinning can produce a porous fiber structure with good mechanical properties. This technique can produce a fibrous scaffold that similar to collagen fibers in the extracellular matrix of the meniscus (Platt, 2005). The high surface to volume ratio and porosity generated by electrospun nanofibers facilitate cell attachment and cell proliferation scaffolds (Kumbar *et al.*, 2008). Additionally, a high tensile strength scaffold can be obtained from this method. Unlike an articular cartilage, when compressive force passes through the meniscus in vertical direction, the meniscus transforms this load into a hoop stress. Circumferential fibers of collagen therefore withstand the tensile stress (Rothrauff *et al.*, 2016). As aforementioned, our research aims to develop a good cell adhesive and high tensile strength scaffold. The effect of SF/PVA ratios on the nanofiber characteristic (morphology, wettability and mechanical properties) was determined.

Materials and Methods

Materials

Silk cocoon (*Bombyx mori*) was obtained from Queen Sirikit Sericulture Center, Nakhon Ratchasima, Thailand. The chemical, sodium carbonate (Na_2CO_3), calcium chloride ($\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$) and 99% formic

acid were purchased from Erba Carlo, Italy. Poly (vinyl alcohol) (PVA) (Mw 146,000-186,000, 87-89% hydrolyzed) was purchased from Sigma Aldrich, Australia.

Preparation of Regenerated SF

Silk Fibroin (SF) was extracted by boiling in 1%w/v Na₂CO₃ for 20 min and washed with deionize water in order to remove sericin. The degummed silk was dried at 105°C for 3 h. Then, SF was dissolved in 46% w/v CaCl₂·6H₂O and heated at 100±2°C for 1 h. Then, the solution was dialyzed with deionized water. After dialysis, SF solution was freeze dried at -40°C.

Electrospinning of Blended SF/PVA

Spinning solution was prepared by dissolving 12% w/v SF in formic acid and 10% w/v PVA in deionize water. The PVA solution was stirred at 80°C until the solution was clear. Next, SF solution mixed with PVA solution and stirred for 2 h to obtain a homogeneous solution. The ratio between SF/PVA solution was varied as shown in Table 1. The blended solution was loaded into a 10 mL syringe with 20 gauge blunt tip needle. The electrospinning was conducted at the voltage setting at 20 kV and flow rate of 0.5 mL/h with a tip to accumulator distance of 10 cm.

Table 1. Composition of SF blend with PVA (SF/PVA)

Sample	SF (mL)	PVA (mL)	Ratio
a	100	0	SF/PVA 100:0
b	75	25	SF/PVA 75:25
c	50	50	SF/PVA 50:50
d	25	75	SF/PVA 25:75
e	0	100	SF/PVA 0:100

Characterization of Blended Electrospun Nanofiber

Morphology

Fiber diameter of the nanofiber sheath were observed by Field Emission Scanning Electron Microscope (FE-SEM) (Zeiss AURIGA FE-SEM/FIB/EDX). The nanofiber was selectively measured in different surface area were investigated.

Contact Angle Test

The electrospun nanofiber was observed using contact angle. The water was dropped onto the surface of the nanofiber with a needle syringe. Images of the drop was captured after the drop set onto the nanofiber. The contact angle was calculated by the software image J for analyzing the shape of the drop.

Fourier Transform Infrared Spectroscopy

Fourier transform infrared spectroscopy (FT-IR) (Bruker Tensor27) was used to identify the functional groups of SF/PVA nanofibrous scaffold. The spectra were recorded over a wave number range of 4,000-400 cm⁻¹ at resolution of 4 cm⁻¹.

Thermogravimetric Analysis

SF/PVA nanofiber was characterized by the TGA instrument (model STA 6000, Perkin-Elmer) in non-isothermal mode. The heating rate was 10°C/min under an inert nitrogen atmosphere over the temperature range of 50-700°C.

Tensile properties

The nanofibrous scaffold was cut into 1.5 cm in width, 10 cm in length and 0.02 mm in thickness tensile test was done according to ASTM D882 by using a universal testing machine (Model 4502, Instron, Norwood, MA) with a load cell of 5 kN.

Results and Discussion

Nanofiber Morphology

Figure 1 shows the field emission scanning electron microscope images of SF/PVA blended nanofiber at various ratio between SF to PVA including 100:0, 75:25, 50:50, 25:75, and 0:100. Each nanofiber shows a different shape and diameter size. The blended SF/PVA of 100:0 with diameter size in range of 34±11 nm. contained beaded formation on nanofiber. The beads formation may be caused by low molecular weight, charge density, or high surface tension. The morphology of SF/PVA at ratio of 75:25, 50:50, and 25:75 showed an interconnecting network structure on the fiber surface and bead free, which their mean diameter were 54±17, 62±21, and 74±18 nm, respectively. Whereas, blended SF/PVA of 0:100 showed random and bead free nanofiber with smooth surface, and diameter size in range of 183±31 nm. However, on blending SF and PVA, the nanofiber diameter was increased when SF content was decreased. The small diameter of SF/PVA nanofiber led to an increase in surface area. This interconnecting network structure on fiber surface of the scaffold nanofiber will play an important role in tissue engineering. In terms of a place for good cell attachment, cell proliferation, and better nutrient diffusion (Pillai *et al.*, 2016). In this study, the morphology of nanofiber from FE-SEM shows the appearance of heterogeneities in the structure that may have significant impact on behaviour of nanofibrous material in medicinal applications, for example cell culture or drug release (Sirc *et al.*, 2012).

Contact Angle

Hydrophilicity is an important role for biocompatibility of scaffold for tissue engineering. The hydrophilicity of the different blended nanofiber was shown in Figure 2 and Table 2.

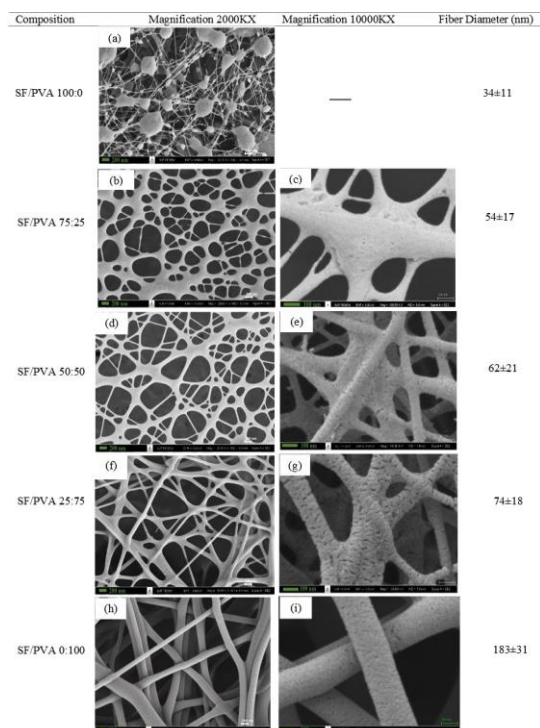


Figure 1. FE-SEM image of SF/PVA nanofiber various ratio and different magnification; (a) SF/PVA 100:0, (b) and (c) SF/PVA 75:25, (d) and (e) SF/PVA 50:50, (f) and (g) SF/PVA 25:75, (h) and (i) SF/PVA 0:100

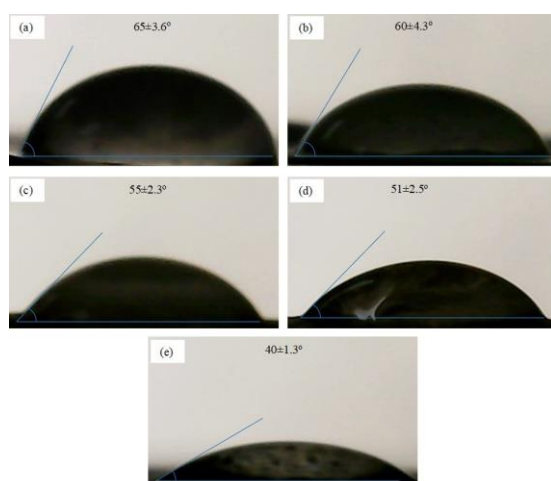


Figure 2. Contact angle of SF/PVA nanofiber various ratio; (a) SF/PVA 100:0, (b) SF/PVA 75:25, (c) SF/PVA 50:50, (d) SF/PVA 25:75, and (e) SF/PVA 0:100

Contact angle of the SF/PVA nanofiber showed the value in range of 40°-65°. The increase in contact angle was observed with an increase SF content. This is due to the the hydrophobicity of the SF/PVA nanofiber. Normally, SF is mostly composed of hydrophobic amino acids domains of the H-chain that contains a repetitive hexapeptide sequence of Gly-Ala-Gly-Ala-Gly-Ser and repeat units of Gly-Ala/Ser/Tyr dipeptides. The hydrophobic amino acid domains of H-fibroin fold and bond together via hydrogen bonds, Van der Waals forces, and hydrophobic interactions, to form anti-parallel β -sheet crystalline structures. (Qi *et al.*, 2017; Nguyen *et al.*, 2019). This structural characteristic provides enough hydrophobicity to increase contact angles of the SF/PVA nanofiber. Polymers with an air-water contact angle around 60°-65° leads to cell attachment and cell proliferation (Bhattacharjee *et al.*, 2015). The highest level of cell attachment (NIH 3T3 fibroblasts) at hydrophilic surfaces was obtained from nanofiber with contact angles in the range of 20°-60° (Wei *et al.*, 2020). Therefore, the hydrophilicity of nanofiber prepared from our study was suitable for scaffold tissue engineering application.

Fourier Transform Infrared (FT-IR) Analysis

FT-IR spectra of the different nanofiber was investigated as shown in Figure 3. The main characteristic peaks of SF contain conformation of β -sheet structure which conformation shows

Table 2. Contact angle and Tensile properties of SF/PVA nanofiber various ratio

Composition	Contact angle (°)	Ultimate Tensile Strength (MPa)	Elongation at Break (%)
SF/PVA 100:0	63±3.6	-	-
SF/PVA 75:25	60±4.3	0.94±0.06	10.8±1.1
SF/PVA 50:50	55±2.3	9.45±2.44	39±4.8
SF/PVA 25:75	51±2.5	5.78±0.92	35.3±11
SF/PVA 0:100	40±1.3	3.93±0.64	171±33

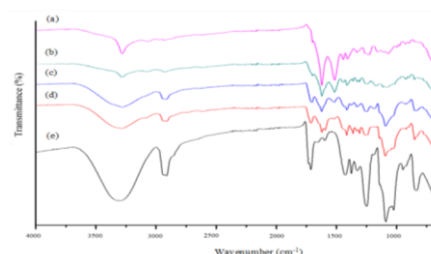


Figure 3. FT-IR spectra of SF/PVA nanofiber various ratio (a) SF/PVA 100:0, (b) SF/PVA 75:25, (c) SF/PVA 50:50, (d) SF/PVA 25:75, and (e) SF/PVA 0:100

characteristic peaks; absorption frequencies to β -sheet form at $1,625\text{--}1,620\text{ cm}^{-1}$, $1,510\text{--}1,500\text{ cm}^{-1}$, and $1,245\text{--}1,240\text{ cm}^{-1}$, representing amide I, amide II, and amide III, respectively. The amide I band mainly comes from C=O stretching with minor contributions from N-H in-plane bending while the amide II band is mainly caused by C-N stretching and N-H in-plane bending of the SF backbone (Ling *et al.*, 2013). The spectrum of PVA was obtained at $3,300\text{ cm}^{-1}$ (-OH stretching), $2,960\text{--}2,920\text{ cm}^{-1}$ (-CH stretching) and $1,750\text{--}1,720\text{ cm}^{-1}$ (-C=O stretching), $1,470\text{--}1,450\text{ cm}^{-1}$ (CHOH bending), $1,250\text{ cm}^{-1}$ (C-O stretching), and $1,090\text{--}1,080\text{ cm}^{-1}$ (C-O out-of-plane bending), respectively (Bhattacharjee *et al.*, 2015). These bands assigned to PVA became stronger with increasing PVA contents. The characteristic absorption bands of SF/PVA blended nanofiber showing the wave number ranges of amide I, amide II, and amide III were shifted from $1,625\text{--}1,620\text{ cm}^{-1}$ to $1,630\text{--}1,627\text{ cm}^{-1}$, $1,510\text{--}1,500\text{ cm}^{-1}$ to $1,515\text{--}1,512\text{ cm}^{-1}$, and $1,245\text{--}1,240\text{ cm}^{-1}$ to $1,250\text{--}1,248\text{ cm}^{-1}$, respectively. Furthermore, the width of the OH region $3,350\text{--}3,300\text{ cm}^{-1}$ was extended by blending SF and PVA which could characterize the increase of the intensity of hydrogen bonding in FTIR spectra (Niu *et al.*, 2019). This may confirmed the chemical interactions between the amino group of silk fibroin and the hydroxyl group of polyvinyl alcohol. However, it hardly prove the extent of the SF/PVA chemical interactions. This is because the moisture product impurity can also give rise to the broadened IR signal between the $3,350\text{--}3,300\text{ cm}^{-1}$ region.

Thermal Stability

Figure 4 shows the thermal stability of SF/PVA at various ratios. The TGA thermogram of all electrospun nanofiber showed the first step of weight loss around $50\text{--}180^\circ\text{C}$ caused by the evaporation of water molecules in fiber about 4%. Thermograms of SF/PVA 100:0 and SF/PVA of 0:100 showed the second step decomposition around $200\text{--}360^\circ\text{C}$ and $290\text{--}350^\circ\text{C}$, respectively. Decomposition of SF may be related with the breakdown of side-chain groups of amino acid residues as well as the cleavage of peptide bonds of silk fibroin (Raksa *et al.*, 2020) and degradation of PVA that is an amorphous polymer. The third step after 350°C is the degradation of the crystalline region in PVA. The second degradation step of SF/PVA blended nanofiber around $200\text{--}380^\circ\text{C}$ may be attributed to the major composition of the silk protein. The third step showed degradation of polymer chain above 390°C . When SF content was increased, it causes an increase in the thermal stability of the blended nanofiber. Usually, sterilization is an important for scaffold and the

suggestion for sterilization in an autoclave use steam heated to 121°C or 134°C . To achieve sterility, a holding time of at least 15 min at 121°C or 3 min at 134°C is required (Baume *et al.*, 2016). The thermal stability of the prepared SF/PVA nanofiber in this study was therefore suitable with sterilization in an autoclave.

Tensile Properties

Mechanical properties of SF/PVA nanofiber at different ratio were shown in Table 1 and stress-strain curves of all nanofiber were shown in Figure 5. The result of SF/PVA 0:100 nanofiber exhibited relatively low mechanical strength. The SF/PVA of 50:50 showed higher tensile strength than another nanofiber. Silk fibroin showed an improvement in the tensile strength of the electrospun fibrous scaffold. These improvement in mechanical properties is attributed to well oriented β -sheet crystallite structure and the shear alignment of the fiber chains (Altman *et al.*, 2003). This is essential property for the meniscus scaffold for tissue engineering. However, when SF content was increased to very high ratio (SF/PVA 75:25); the SF/PVA blended nanofiber was more difficult to be processed and nonuniform thickness of the nanofibers were obtained with the lowest tensile strength and elongation at break.

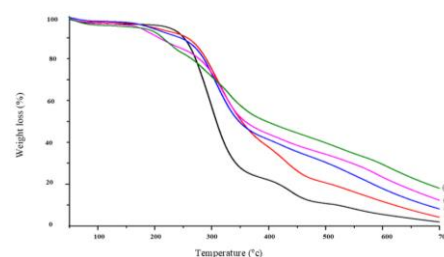


Figure 4. TGA thermogram of SF/PVA nanofiber various ratio; (a) SF/PVA 100:0, (b) SF/PVA 75:25, (c) SF/PVA 50:50, (d) SF/PVA 25:75, and (e) SF/PVA 0:100

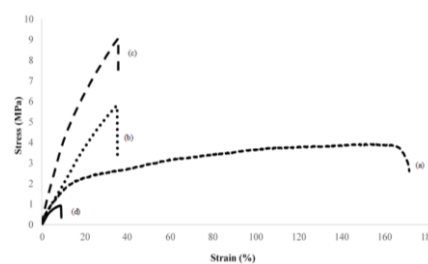


Figure 5. Stress-Strain curve of SF/PVA nanofiber various ratio; (a) SF/PVA 0:100, (b) SF/PVA 25:75, (c) SF/PVA 50:50, and (d) SF/PVA 75:25

Conclusions

In this study, we have successfully fabricated a nanofibrous scaffold from silk fibroin and polyvinyl alcohol by electrospinning technique. It was found that SF has a direct impact on modifying the fiber morphological properties, wettability, and tensile properties. Specifically, the tensile strength of SF/PVA nanofiber was improved with increasing SF content. The chemical interactions between the amino group of silk fibroin and the hydroxyl group of polyvinyl alcohol were confirmed by FTIR spectra. The thermal stability of the prepared the blended nanofiber was suitable with sterilization.

Acknowledgement

This work was financially supported by Rajamangala University of Technology Isan, Nakhon Ratchasima, and Suranaree University of Technology, and Science, Research and Innovation Promotion Fund from Thailand Science Research and Innovation (TSRI).

References

- Abrams, G.D., Frank, R.M., Gupta, A.K., Harris, J.D., McCormick, F.M., and Cole, B.J. (2013). Trends in meniscus repair and meniscectomy in the United States, 2005-2011, *Amer. J. Sport. Med.*, 41(10):2,333-2,339.
- Agarwal, S., Wendorff, J.H., and Greiner, A. (2008). Use of electrospinning technique for biomedical applications. *Polymer*, 49:5,603-5,621.
- Altman, G.H., Diaz, F., Jakuba, C., Calabro, T., Horan, R.L., Chen, J., H. Lu, Richmond, J., and Kaplan, D.L. (2003). Silk-based biomaterials. *Biomater.*, 24(3):401-416.
- Baker, B.E., Peckham, A.C., Puppato, F., and Sanborn, J.C. (1985). Review of meniscal injury and associated sports, *Am. J. Sports Med.*, 13:1-4.
- Baume, A.S., Boughton, P.C., Coleman, N.V., and Ruys, A.J. (2016). Sterilization of tissue scaffolds. *Characterisation and Design of Tissue Scaffolds*. Woodhead Publishing, Sawston, UK, p. 225-244.
- Bhattacharjee, P., Kundu, B., Naskar, D., Maiti, T.K., Bhattacharya, D., and Kundu, S.C. (2015). Nanofibrous nonmulberry Silk/ PVA scaffold for osteoinduction and osseointegration. *Biopoly.*, 103(5):271-284.
- Campbell, S.E., Sanders, T.G., and Morrison, W.B. (2001). Mr imaging of meniscal cysts: Incidence, location, and clinical significance. *AJR Am. J. Roentgeno.*, 177:409-413.
- Chang, A., Moio, K., Chmiel, J.S., Eckstein, F., Guermazi, A., Almagor, O., Cahue, S., Wirth, W., Prasad, P., and Sharma, L. (2011). Subregional effects of meniscal tears on cartilage loss over 2 years in knee osteoarthritis. *Annals of the Rheumatic Disease.*, 70(1):74-79.
- Chen, M., Gao, S., Wang, P., Li, Yan., Guo, W., Zhang, Y., Wang, M., Xiao, T., Zhang, Z., Zhang, X., Jing, X., Li, X., Liu, S., Guo, Q., and Xi, T. (2018). The application of electrospinning used in meniscus tissue engineering. *J. Biomater. Sci., Poly. Ed.*, 29(5):461-475.
- Englund, M., Guermazi, A., Roemer, F.W., Yang, M., Zhang, Y., Nevitt, M.C., Lynch, M., J.A., Lewis, C.E., Torner, J., and Felson, D.T. (2010). Meniscal pathology on mri increases the risk for both incident and enlarging subchondral bone marrow lesions of the knee. *Annals of the Rheumatic Diseases*, 69(10):1,796-1,802.
- Gunn, J. and Zhang, M. (2010). Polyblend nanofibers for biomedical applications: Perspectives and challenges. *Trend. Biotech.*, 28(4):189-197.
- Halili, N.A. (2011). Collagen-based meniscus tissue engineering: design and application, Thesis, [Ph.D. thesis, Doctor of Philosophy in Department of Biotechnology]. Middle East Technical University, Turkey.
- Hede, A., Jensen, D.B., Blyme, P., and Sonne-Holm, S. (1990). Epidemiology of meniscal lesions in the knee. *Acta Orthop Scand.*, 61:435-437.
- Huang, W., Ling, S., Li, C., Omenetto, F.G., and Kaplan, D.L. (2018). Silkworm silk-based materials and devices generated using bio-nanotechnology. *Chem. Soc. Rev.*, (47):6,486-6,504.
- Kumbar, S.G., James, R., Nukavarapu, S.P., and Laurencin, C.T. (2008). Electrospun nanofiber scaffolds: Engineering soft tissues, *Biomed. Mater.*, 3(3):034002.
- Kumkun, P., Tuancharoensri, N., Ross, G., Mahasaranon, S., Jongjitwimol, J., Topham, P.D., and Ross, S. (2019). Green fabrication route of robust, biodegradable silk sericin and poly (vinyl alcohol) nanofibrous scaffolds. *Poly. Int.*, 68(11):1,903-1,913.
- Ling, S., Qi, Z., Knight, D.P., Shaoa, Z., and Chen, X. (2013). FTIR imaging, a useful method for studying the compatibility of silk fibroin-based polymer blends. *Polym Chem.*, 4:5,401-5,406.
- Nguyen, T.P., Nguyen, Q.V., Nguyen, V-H., Le, T-H, V., Huynh, Q.N., Vo, D.V.N., Trinh, Q.T., Kim, S.Y., and Van Le, Q. (2019). Silk fibroin-based biomaterials for biomedical applications: a review, *Polymers*, 24; 11(12):1933.
- Niu, C., Li, X., Wang, Y., Liu, X., Shi, J., and Wang, X. (2019). Design and performance of a poly(vinyl alcohol)/silk fibroin enzymatically crosslinked semi-interpenetrating hydrogel for a potential hydrophobic drug delivery. *Royal Soc. Chem.*, 9:41074.
- Panilaitis, B., Altman, G.H., Chen, J., Jin, J.H., Karageorgiou, V., and Kaplan, D.L. (2003). Macrophage responses to silk. *Biomater.*, 24(18):3,079-3,085.
- Pillai, M.M., Gopinathan, J., Indumathi, B., and Manjoosha, Y.R. (2016). Silk-pva hybrid nanofibrous scaffold for enhanced primary human meniscus cell proliferation. *J. Membrane Biol.*, 249(6):813-822.
- Platt, M.A. (2005). Tendon repair and healing. *Clin. Podiatr. Med. Surg.*, 22:553.
- Qi, Y., Wang, H., Wei, K., Yang, Y., Zheng, R.Y., Kim, S., and Zhang, K.Q. (2017). A review of structure construction of silk fibroin biomaterials from single structures to multi-level structures. *J. Mol. Sci.*, 18(3):237.
- Raksa, A., Utke, R., Ruksakulpiwat, C., Numpaisal, P., and Ruksakulpiwat, Y. (2020). Morphological and chemical characterization of electrospun silk fibroin/polyvinyl alcohol nanofibers. *AIP Conference Proceeding*, 2279:080004-1.
- Roos, H., Lauren, M., Adalberth, T., Roos, E.M., Jonsson, K., and Lohmander, L.S. (1998). Knee osteoarthritis after meniscectomy: Prevalence of radiographic changes after twenty-one years, compared with matched controls. *Arthritis Rheum.*, 41(4):687-693.
- Ross, S., Yooyod, M., Limpeanchob, N., Mahasaranon, S., Suphrom, N., and Ross, G.M. (2017). Novel 3D porous semi-IPN hydrogel scaffolds of silk sericin and poly(N-hydroxyethyl acrylamide) for dermal reconstruction. *Express Poly. Lett.*, 11(9):719-730.

- Rothrauff, B.B., Numpaisal, P., Lauro, B.B., Alexander, P.G., Debski, R.E., Musahl, V., and Tuan, R.S. (2016). Augmented repair of radial meniscus tear with biomimetic electrospun scaffold: an in vitro mechanical analysis. *J. Experim. Orthop.*, 3(23):1-10.
- Sakunphanitphan, S., Jantarat, J., Sritanaudomchai, H., Oonkhanond, B., and Hargreaves, K.M. (2019). The effect of silk fibroin hydrogel on proliferation of human stem cells from the apical papilla. *M. Dent. J.*, 39(1):35-40.
- Sill, T.J. and Von Recum H.A. (2008). Electrospinning: Applications in drug delivery and tissue engineering. *Biomater.*, 29(13):1,989-2,006.
- Sirc, J., Hobzov, R., Kostina, N., Munzarov, M., Juklickov, M., Lhotka, M., Kubinov, S., Zajicov, A., and Michalek, J. (2012). Morphological characterization of nanofibers: methods and application in practice. *J. Nanomat.*, p. 1-14.
- Su, D., Ding, S., Shi, W., Huang, X., and Jiang, L. (2019). Bombyx mori silk-based materials with implication in skin repair: Sericin versus regenerated silk fibroin. *J. Biomat. Appl.*, 34(1):36-46. DOI: <https://doi.org/10.1177/0885328219844978>
- Tarun, G., Ajay, B., Bhawna, K., Sunil, K., and Ravi, J. (2011). Scaffold: tissue engineering and regenerative medicine. *Int. Res. J. Pharm.*, 2(12):37-42.
- Thompson, C.J., Chase, G.G., Yarin, A.L., and Reneker, D.H. (2007). Effects of parameters on nanofiber diameter determined from electrospinning model. *Poly.*, 48(23):6,913-6,922.
- Wei, Z., Gua, J., Yea, Y., Fanga, M., Langa, J., Yanga, D., Pan, Z. (2020). Biodegradable poly(butylene succinate) nanofibrous membrane treated with oxygen plasma for superhydrophilicity. *Surf. Coat. Technol.*, 381:1-7.