e-ISSN:2321-6212
p-ISSN:2347-2278
Research and Reviews: Journal of Material Sciences

# Modification of Fibroins: Approaches to Get Optimise Scaffolds for Musculoskeletal Tissue Engineering.

Patitapabana Parida[1*], Niraj Babu[1], Bibhukalyan Prasad Nayak[1], Rohit Raj[2], and Ajit Behera[3]

[1]Department of Biotechnology & Medical Engineering, National Institute of Technology, Rourkela, Odisha-769008, India.
[2]Department of Biotechnology, National Institute of Technology, Raipur, Chhattisgarh - 492010, India.
[3]Department of Metallurgical & Materials Engineering, Indian Institute of Technology, Kharagpur-721302, India.

Research Article

Received: 05/08/2013
Revised: 11/09/2013
Accepted: 22/09/2013

***For Correspondence**

Department of Biotechnology & Medical Engineering, National Institute of Technology, Rourkela, Odisha-769008, India.

**Keywords:** Muskuloskletal system, Tissue engineering, Silk, Electrospinning, crosslinking.

## ABSTRACT

The components of musculoskeletal system that consists of bone, ligament tendons and muscles are most vulnerable to injury from sports and related activities. Replacing the injured tissues more specifically the bone is a challenge to tissue engineers. Aiming at regeneration of bone tissues, silk fibroin from *Bombyx mori, a* novel biocompatible natural polymer has been explored either alone or as organic/inorganic composites for optimal growth of bone cells or differentiation of stem cells to bone cells *in vitro*. In the process, raw silk fibroins need to be were degummed, usually by alkali treatment method, to ameliorate the cytotoxicity from sericin. Most recently, the nanofibers of silk from electrospinning has shown a promise for bone tissue engineering. To get nanofibers, the degummed silk is usually dissolved in compatible solutions i.e. $CaCl_2$ in $H_2O$ or LiBr in $C_2H_5OH$ at different concentrations followed by dialysis to remove toxic ions. The ion free silk solution can be Electrospun alone or can be blended with various biopolymers for the fabrication of nanofibre biodegradable composite. Other approaches like crosslinking an array of biopolymers with silk or freeze drying the silk fibroins are also in pipeline to develop a novel silk based scaffold for bone tissue engineering.

## INTRODUCTION

Tissue engineering scaffolds should mimic in piece the extracellular matrix (ECM) structure and biological function [1].Natural protein silk hasbrilliant biocompatibility, remarkable mechanical property as well as tailorable degradability[2]. Tissue engineering has emerged as an excellent approach for the damaged tissue repair/regeneration , by means of the potential to circumvent all the limitations of autologous and allogenic tissue repair[3]. Silkworm Bombyxmoriderived silk filament is a usual protein mainly made of sericin (the outer coating) and fibroin (the inner brins). The sericin protein is removed by a process called degumming in manufacturing, so that the expression silk is normally improperly used to characterize only one of its two components, the silk fibroin[4]. In addition to the imposing mechanical properties, silk fibroin is also a degradable material. Extremely crystallized silk degrades little by little,but the rate in vivo depends on the implantation site & mechanical potency [5]. Degumming with $Na_2CO_3$ solution had the ceiling impact on silk fibroin fiber, ensuing in significant reduction of the thermal stability and crystallinity, and the fiber tensile property was only half of that degummed with urea buffer. The moderate degumming solution was urea buffer, followed by powerfully alkaline electrolyzed water (SAEW)[6]. For degumming several alkalis such as NaOH(Sodium hydroxide) or $Na_2CO_3$ (Sodium carbonate) are often used nowadays. However, these y alkali treatments impose a relatively unsympathetic irritation to silk fibroins[7].

## MATERIALS AND METHODS

For the groundwork of Bombyxmori Silk with Poly(ethylene oxide),B. morisilk fibroin were 30 min boiled in an 0.02 M $Na_2CO_3$aqueous solution and the extracted silk was then dissolved in 9.3 M LiBr solution at 600C and

dialyzed in water using a Slide-a-Lyzer dialysis. by adding PEO directly into the silk aqueous solutions generating Silk/PEO blends in water were prepared [8].

For the Silkbased fibroin and collagen preparation for vascular tissue productioncocon was boiled in 0.5 wt% $Na_2CO_3$ aqueous solutions for 1 h and then degummed SF was dissolved in $CaCl_2/H_2O/C_2H_5OH$ solution (1/8/2 in mol ratio) for 40 min at 80 ◦C, followed by 3 days dialysis using cellulose dialysis . Then the HAc solution of collagen was added to the SF aqueous solution to set up SF/Col blend solution with a collagen content of 10. The preparation of mixed solutions all involved gentle stirring and heating. The stirring temperature was controlled between 45 and 55 ◦C. To avoid gelation, the prepared solution was preserved at 4◦C before electrospinning[9].

For the Investigation of Nanofiber Morphology and Process Optimization using Response Surface Methodology B. mori cocoons were de-gummed for 1 h with 2 g/l $Na_2CO_3$ solution and 1 g/l commercial anionic detergent at 100oC and followed by warm distilled water rinsing in order to removeSericin from the silk fibers surface. Degummed silk fibroin (SF) was first dissolved in a ternary solvent system of $CaCl_2/CH_3CH_2OH/H_2O$ (1:2:8 in molar ratio) at 70 oC for 4 h. After 3 days dialysis with cellulose tubular membrane in distilled water, the silk fibroin solution was lyophilized to obtain the regenerated SF sponges. The regenerated SF sponge was dissolved in 98 % formic acid for 30 min. to prepare 8-14 % (W/V) SF/formic acid solutions[10].

Silk Fibroin Scaffolds for Ligament Tissue Engineering Applications:Silk fibroin will have to be dissolved in solvents of hexafluoroisopropanol (HFIP), water, and a combination of 50/50 HFIP/water ratio in order to resolve variance between solvent properties used to create the scaffolds. Currently organic solvents and there combinations are being used to liquefy polymers in solution prior toelectrospinning. Electrospinningfrom an organic solvent, water, and a 50/50 fraction of the organic solvent and water will create scaffolds that can be examined for average porosity, permeability and fiber diameter, and for mechanical properties [9].

For non-woven Silk Fibroin Scaffold preparation Raw B. mori silk fibers were boiled for half an hour in a 0.5% $Na_2CO_3$, and rinsed thoroughly with water to remove the glue-like sericin proteins surrounding the fibroin filaments and dried in air. Degummed silk fibers were soaked into test tube containing the 98% formic acid solution and 0.01 w/v% calcium chloride (material-to-fluid ratio, 1:200) at room temperature. The fiber suspension was shaken for 30min to attain homogenous fiber distribution and kept still for 24 h. Finally, the acid solution was evaporated through water bath at 40 °C in an aerator, and the resulting non-woven material was repeatedly washed with distilled water to remove any residual salt and vacuum dried. The degummed silks were completely dissolved after being soaken in a solution of calcium chloride/ethanol/ distilled water (1:2:8 mole ratio) at 80 °C for 4 h through stirring. The prepared solution was purified by being dialyzed against distilled water for 3 days. 20mL of fibroin solution (V1) with a concentration of 2.5 w/v% (C1) was held in a graduated flask. Finally, silk fibroin solution with various concentrations of 0.75, 1.5, 3, 6, 9 and 12 w/v% were prepared by dilution or evaporation.Next, samples were frozen for 6 h at the temperature of −80 °C and vacuum dried for 48 h in a freeze dryer[10].

For the Macro/microporous silk fibroin scaffolds preparation with potential for articular cartilage and meniscus tissue engineering applications,cocoons were boiled for 1 h in an aqueous $Na_2CO_3$solution (0.02 M) and then rinsed thoroughly with distilled water in order to extract the glue-like protein sericin and wax. The purified silk fibroin was dissolved for 1h in 9.3 M LiBr solution at 700 C, yielding a 16% (w/v) solution and then dialyzed for 48h in distilled water using a benzoylated dialysis tubing. Next, the silk fibroin aqueous solution was dialyzed against a 20 wt.% poly(ethylene glycol) solution for 6 h. The prepared concentrated silk fibroin solution was diluted to 8, 10, 12 and 16 wt. %, respectively. In the case of the preparation of scaffolds from the 12 and 16% silk fibroin solutions, the $Na_2CO_3$particles were slowly added to the silicon tubing, which was gently tapped to facilitate the precipitation of the salt particles. Following this, the silicon tubing was placed in a Petri dish and dried at room temperature for 48 h. In order to extract the $Na_2CO_3$, the tubing was immersed in distilled water for 3 days. Finally, the scaffolds were obtained by using a stainless steel punch (inner diameter: 6 mm) in order to remove the outer skin that is generated, followed by 1 day freezingat 80oC and freeze[11].

For producing Control of Silk-Based ElectrospinningCocoons were boiled for 20 min in an 0.02 M $Na_2CO_3$ aqueous solution and then rinsed thoroughly with distilled water to remove the sericin proteins. After drying, 13.5 g extracted silk fibroin was dissolved in 50 mL LiBr solution (9.3 M) at 60 °C for 4 h,yielding a 20% (w/v) solution. This solution was dialyzed against distilled water using Slide-a-Lyzer dialysis cassettes for 72 h to remove the salt. The solution was optically clear after dialysis and was centrifuged to remove the small amount of silk aggregates that formed during the process. To prepare silk films containing different nanostructures the silk solution (4 wt %) was cast on polystyrene Petri dishes. Silk films mainly composed of nanospheres were prepared directly by drying silk solution within 12 h, while silk films mainly composed of nanofilaments formed with a slower drying time of 4 days, according to our previous procedures[12].

For architecture of silk based Nanofibrous fibroin scaffolds Cocoons were boiled for 20 min in an 0.02M $Na_2CO_3$ aqueous solution , and then rinsed thoroughly with distilled water to remove sericin proteins and thenthe

extracted silk was dissolved in 9.3M LiBr solution at 60°C for 4h, yielding a 20wt% solution and dialyzed for 72h in distilled water using Slide-a-Lyzer dialysis cassettes. The collagen solution and silk solution was blended at 4°C using different collagen and silk contents by changing the volume ratio. In order to achieve scaffolds with various pore sizes, water was added into the blend solutions to adjust the concentrations of silk and collagen. The aqueous blend of silk-collagen solutions with different concentrations and ratios of silk and collagen were directly placed at -20°C for about 12 h to freeze them and then lyophilized for about 48h. As controls, silk scaffolds were also prepared by salt-leaching[13].

For optimization strategies for electrospun silk fibroin tissue engineering scaffoldsB. moricocoons were boiled in 0.02 M $Na_2CO_3$ aqueous solution, rinsed with ultrapurified water (UPW) and dissolved in 9 M LiBr at 55oC to generate a 10% w/v solution. This solution was dialyzed against UPW for 48 h. After desalination a second dialysis step against PEG 6000 (200 g/1.5 l UPW) was performed to generate a SF solution of higher concentration. SF solution of 12.5% w/w was obtained by diluting the concentrated SF solution with UPW. A SF/PEO blend was used for electrospinning to enable stable and continuous spinning. 2 ml of PEO solution (5% w/w) was mixed with 5 ml of SF solution (12.5%) by moderate stirring for further use in the electrospinning process[14].

Preparation of electrospun silk fibroin fiber mats as bone scaffolds, Thai silkworm race having yellow cocoons, whereas DOAE-7 is a Chinese/Japanese hybrid silkworm race having white cocoons. It should be noted that DOAE-7 is a hybrid silkworm race developed by Thailand. Both of these races are famous for their high quality yarns. Both types of cocoons were first boiled in water and then dried at 60 ◦C for 24 h in an oven to obtain raw silk fibers. These fibers were de-gummed three times with 0.5% (w/v) $Na_2CO_3$ solution at 100◦C for 30 min and then rinsed with warm water. De-gummed silk was dissolved in a ternary solvent system of $CaCl_2$/ethanol/$H_2O$ in a 1:2:8 mol ratio at 70 ◦C. After 3 days dyalised, the obtained SF solution was filtered and lyophilized to obtain SF sponges. The SF solutions for e-spinning were finally prepared by dissolving weighed amount of SF sponges in 85% formic acid at various concentrations ranging from 10 to 40% (w/v) with 5% (w/v) increment[15].

For production of silk sericin/silk fibroin blend Nanofibers, SS solution (20 wt.%) and SF solution (10 wt.%) were prepared by stirring the samples in trifluoroacetic acid (TFA) at 25°C for 3 h. Solutions of 0.45, 0.3, and 0.15 g SS were mixed with 0.3, 0.6, and 0.9 g SF solutions respectively, so that the SS/SF (w/w: 75/25, 50/50, and25/75) blend solutions were prepared. The SS/SF (75/ 25) blend nanofibers were produced from solutions containing 75 wt. % SS and 25 wt. % SF. The pure and mixed solutions were stirred for 3 h and stored in the refrigerator (4°C) for 12 h, while theelectrospinning solution was prepared[16].

For the Quantifying Osteogenic Cell Degradation of Silk Biomaterials, Cocoons of B.mori were 30min boiled in 0.02 M $Na_2CO_3$ aqueous solution and then rinsed thoroughly with distilled water to eliminate the glue-like sericin proteins. The extracted silk fibroin was dissolved in 9.3 M LiBr solution at 60 °C for 4 h, yielding a 20 w/v% solution and was dialyzed for 2days in distilled water using a Slide-a-Lyzer dialysis cassette.13 The final concentration of silk fibroin aqueous solution was 8% w/v. Patterned polydimethylsiloxane substrates of 2-3 mm thickness were prepared by casting on 1200 lines/mm (blaze angle 17°/271/2) diffraction grating surfaces. PDMS rounds were punched with 11 mm diameters. The PDMS substrates were placed casting by a 70% ethanol wash with three DI water washes. A 62 µL aliquot of 8% silk solution was cast on the grooved PDMS substrates to generate a 50 µm thick film.22. By drying overnight,a waterannealing process was performed by placing the silk film within a waterfilled desiccator at 24 mmHg vacuum for a 5 h period[17].

De novo engineering of reticular connective tissue in vivo by silk fibroin nonwoven materials, Raw silk fibers from B.mori cocoons were boiled for 1 h in a 0.7% w/v soap solution, and then rinsed thoroughly with distilled water to remove gum like protein sericin that surrounds the fibroin filaments. After drying at room temperature, to remove residual fatty acids the degummed silk fibers were extracted with diethyl ether. SF-based 3D nonwoven substrates were then prepared according to the method of Armato et al. Briefly, degummed silk fibers were soaked at room temperature into 98% formic acid containing 0.01% w/v calcium chloride (material-to-fluid ratio, 1:200)and suspension was shaken for about 30 min to achieve homogeneous fiber distribution. Under this treatment a partial dissolution of SF takes place. The acid solution was evaporated under atmospheric conditions, and the resulting nonwoven material was washed continuously with double distilled water to eliminate any residual salt and finally vacuum dried[18].

In vitro Model of Mesenchymal Condensation DuringChondrogenic Development, aqueous silk fibroin solution was prepared by concentrating 8 wt% solution prepared as described previously [34]. In brief, cocoons of B. mori silkworm silk (Tajima Shoji Co., Ltd., Yokohama, Japan) were boiled for 30 min in 0.02 M $Na_2CO_3$ aqueous solution, and then rinsed thoroughly with distilled water to extract the outer layer of glue-like sericin proteins of silk fibers. The extracted fibroin was dissolved in 9.3M LiBr solution at 60°C for 4 hr, yielding a 20 wt% aqueous solution and dialyzed for 3days against distilled water using Slide-a- Lyzer dialysis cassettes (MWCO 3,500, Pierce) at room temperature for desalination. The dialysate was centrifuged twice, each at -5°C to 10°C for 20 min, to remove impurities and aggregates. The solution obtained from this process was approximately 8 wt% [19].

e-ISSN:2321-6212
p-ISSN:2347-2278

Cytocompatibility of regenerated silk fibroin film: a medical biomaterial applicable to wound healing, the domestic silkworm silk fiber was degummed using $Na_2CO_3$ solution, dissolved in $CaCl_2$-$CH_3CH_2OH$-$H_2O$ (1:2:8 in mole ratio), dialysed, filtered, and dried at 60 °C. In this way, purified regenerated silk fibroin films were obtained. After 60Co-irradiation, sterile regenerated silk fibroin (SF) films, PVC plastic films and PE plastic films were lixiviated in sterile DMEM medium (samples' surface area/medium volume=3 cm2/ml) at 37 °C for 24 h, and then cells of each experimental group were cultivated in the extractions containing 10% FCS. The cells of the control group were cultivated only in complete medium DMEM with 10% FCS[20].

## RESULT AND DISCUSSION

PEO was effectively blended with the fibroin aqueous solution, producing electrospun mats with less than 1 ím diameter fiber was found, with the composition reflective of the solution concentrations. Trditional silk fibroin conformational transitions suggesting that native structural features of the silk were preserved in the electrospinning process were induced with methanol treatment of the fibers.

While acidic solution was inappropriate to be used in the electrospinning because of gelation of the SF/Col solution. In addition, continuous fibers were obtained when solvent was water, while beaded fibers were obtained when HAc solution was used. Too much collagen caused the formation of the belt-like fibers and the slight decrease of the crystallinity. The elongation ratio with addition of 10% collagen decreased, which can be explained by the increase of crystallinity of SF provided promising tubular scaffold.

The electrospinning of silk fibroin was processed and average fiber diameter ranging from 80 nm to 210 nm was obtained depending on the electrospinning condition. While at higher solution concentration uniform and smooth fibers were obtained at the range of the applied voltage examined. The solution concentration was the most significant factor that had considerable effects on the fibers diameter and its standard deviations whereas applied voltage had no significant impact on them. The average fiber diameters and the standard deviation of fiber diameter increased with polymer concentration according to the proposed relationships under the experimental conditions studied in this work [4].

The effects of solvents on electrospun materials has been examined to determine that both the dimensions and secondary structure of silk fibroin nanofibers were affected by the organic solvent chosen to make the solvent solution beforeelectrospinning. The electrospun water solution based scaffolds should equal or exceed the mechanical properties of scaffolds created from HFIP to merit further studies using water as a solvent for electrospinning silk fibroin [5].

With the use of methods to prepare non-woven silk fibroin net and of freeze drying techniques, it is possible to fabricate a 3D porous silk fibroin scaffold with hierarchical fine structure, which may have a potential use as a bioscaffold or other biomaterials [6].

In this study, an initial physicochemical characterization is presented of silk fibroin scaffolds derived from high-concentration aqueous silk fibroin solution and prepared by combining the salt leaching and freeze-drying methodologies. Morphological study revealed that the scaffolds possessed both macro- and microporous structures, and the morphology varied depending on the initial concentration. Micro-CT analysis further demonstrated that the prepared scaffolds possessed high porosity and interconnectivity, which seemed to decrease with increasing silk fibroin concentration. Compressive testing and DMA analysis showed that the mechanical properties of the silk fibroin scaffolds increased vividly with increasing of silk fibroin concentration. Water uptake data demonstrated that the scaffolds presented a large swelling capability that increased with increasing porosity [8].

Silk nanostructure in solution, a key parameter for silk electrospinning, was studied. Nanofilament formation in silk solution increased the spinnability of silk and also improved the control of electrospun fiber diameter. Based on this new mechanism of control of silk solution for spinning, the design and preparation of silk electrospun scaffolds with controllable sizes and properties becomes feasible, which would further facilitate further utility in tissue engineering and drug release systems [9].

Three-dimensional macroporous silk scaffolds with nanofibrous architectures were prepared from silk-collagen aqueous solutions using freeze-drying. By changing the collagen content to control the self-assembly of silk, the nanostructure of the macroporous wall transformed from irregular to nanofibrous structures and then to macroscaled fibrils. The nanofibrous architecture of silk-collagen scaffolds, similar to natural ECM, provided fibroblasts with a favorable microenvironment to augment growth and proliferation [11].

This study explored optimization strategies for scaffold design by introduction and evaluation of topographical, mechanical and chemical cues. We used advanced analytical tools shifting mechanical evaluation from bulk properties down to the singlefiber level. The topography of electrospun scaffolds was impacted by

electrospinning conditions, particularly the rotational speed of the cylindrical target. SF fiber alignment functioned as topographical cue leading to elongated and oriented cellular morphologies and may open an interesting avenue to use SF scaffolds for the de novo engineering of structurally aligned tissues. Fibronectin adsorbed on SF scaffolds was demonstrated by FRET to exhibit partial extension of its dimer arms and functioned as a chemical cue to enhance hMSC adhesion and spreading [12].

At low solution concentrations (i.e., 10–25% (w/v)), e-spinning of both types of SF solutions produced either discrete beads or beaded fibers, while, at high solution concentrations (i.e.,≥30% w/v), only smooth fibers were observed. The average diameter of the e-spun fibers from both types of SF solutions was found to increase monotonically with the increase in the solution concentration, in the range of 217–610 nm (Nang-Lai SF), while that of the fibers from (DOAE-7 SF) solutions being in the range of 183–810 nm. For beaded fibers, an augment in the EFS value caused the number of beads to decrease and the shape of beads to be more elongated, while, for smooth fibers, it was responsible for the observed increase in the fiber diameters [13].

We succeeded in producing SS/SF blend nanofibers by electrospinning with a SS/SF TFA blend solution. The “as-spun” nanofibers exhibited smooth surfaces, round cross sections, and bead-free structures. The average diameters of SS/SF (75/25, 50/50, and 25/75) blend nanofibers were thicker than those of SS or SF nanofibers. The mean diameter of these blended nanofibers decreased, and the number of beads slightly augmented with increasing dissolving time of the SS/SF blend solution prior to electrospinning. The number of beads also augmented with increasing SF content. The SS/SF (100/0, 75/25, and 50/50) blend nanofibers were easily dissolved in water, while the SS/SF (25/75 and 0/100) blend nanofibers were not completely dissolved in water. The SS/SF blend nanofibers were not dissolved in methanol [14].

Osteogenic cells, osteoblasts and osteoclasts, actively degraded silk fibroin protein in vitro, a biomaterial extensively used in bone tissue engineering. The identification of MMP and integrin responses by these cells confirmed the respective contributions to the degradation process [15].

Clearly, while a major in vivo biodegradation of the implanted SF nonwovens remains possible on lengthier terms, the present results imply the notion that biocompatible SF nonwovens are to be integrated, at least provisionally, into the host’s tissues to act there as effective guides for tissue engineering/ regeneration/repair [18].

The intertwinement of nanofibrous scaffold matrix compliance, cell shape, cytoskeletal mechanics, and developmental processes was been addressed by simulating nanofibrillar matrix morphology in the basement membranes. Insight into how polymeric scaffold matrix compliance guides chrondrogenic tissue regeneration was obtained with both human embryonic stem cells and adult stem cells in the presence of chondrogenic soluble factors. This relatively simple nanofiber matrix-based in vitro system allows the study of 3D chondrogenic development [19].

Regenerated silk fibroin film does not have an adverse influence on the growth and biofunction of fibroblasts and vascular endothelial cells. It also does not interfere with the secretion of angiogenesis growth factors such as VEGF, Ang-1, FGF2 and PDGF. Thus, regenerated silk fibroin film is an excellent biomaterial with good biocompatibility [20].

## CONCLUSIONS

Here various types of fabrication were described. In the case of nanofiber the diameter depends on parameter like temperature, humidity, concentration of solution, applied voltage, collector distance, viscosity of materials,. The mechanical strength of nanofibersdepends on the physicochemical properties of polymer. For increment of mechanical strength of silk based nanofiber some other biopolymers were blended during preparation. Other silk based scaffold prepared by lyophilisation& othersolvent evaporation methods the porosity can be enhanced for the utilization in tissue engineering.

## REFERENCES

1. Razdorsky VV. Prospects of using titanium nickelide implants with modified surface in dental implantology. Bull ExpBiol Med. 2008;145(6):758-764.
2. Wang Y, et al. Stem cell-based tissue engineering with silk biomaterials. Biomaterials 2006;27(36): 6064-6082.
3. Vasita R, DS Katti.Nanofibers and their applications in tissue engineering. Int JNanomed. 2006;1(1):15.
4. Wei K, et al. Fabrication of nano-hydroxyapatite on electrospun silk fibroin nanofiber and their effects in osteoblastic behavior. JBiomed Mat Res Part A. 2011;97(3):272-280.
5. Rockwood DN, et al. Materials fabrication from Bombyx mori silk fibroin. Nature Protocols. 2011;6(10):1612-1631.

6. Wang HY, YQ Zhang. Effect of regeneration of liquid silk fibroin on its structure and characterization. Soft Matter. 2013;9(1):138-145.
7. Ho MP, et al. Interfacial bonding and degumming effects on silk fibre/polymer biocomposites. Composites Part B: Engineering. 2012. 43(7):2801-2812.
8. Jin HJ, et al. Electrospinning Bombyx mori silk with poly (ethylene oxide). Biomacromol. 2002;3(6):1233-1239.
9. Zhou J, et al. Electrospinning of silk fibroin and collagen for vascular tissue engineering. Int JBiolMacromol. 2010;47(4):514-519.
10. Amiralian N, M Nouri, MH Kish. An Experimental study on Electrospinning of silk fibroin.
11. Zhao Y, R Legeros, J Chen. Initial Study on 3D Porous Silk Fibroin Scaffold: Preparation and Morphology. Bioceramics Development and Applications. 1.
12. Wei K, BS Kim, IS Kim. Fabrication and biocompatibility of electrospun silk biocomposites. Membranes. 2011;1(4):275-298.
13. Zhang F, et al. Mechanisms and control of silk-based electrospinning. Biomacromol. 2012;13(3):798-804.
14. Liu H, et al. Modification of sericin-free silk fibers for ligament tissue engineering application. J Biomed Mat ResPart B: App Biomat. 2007;82(1):129-138.
15. Lu Q, et al, Nanofibrous architecture of silk fibroin scaffolds prepared with a mild self-assembly process. Biomat. 2011;32(4):1059-1067.
16. Zhang X, et al, Production of silk sericin/silk fibroin blend nanofibers. NanoscaleResLett. 2011;6(1):1-8.
17. Sengupta S, et al. Quantifying osteogenic cell degradation of silk biomaterials. Biomacromol. 2010; 11(12):3592-3599.
18. Dal Pra I, et al. De novo engineering of reticular connective tissue in vivo by silk fibroin nonwoven materials. Biomater. 2005;26(14):1987-1999.
19. Ghosh S, et al. In vitro model of mesenchymal condensation during chondrogenic development. Biomater. 2009;30(33):6530-6540.
20. Liu TI, et al. Cytocompatibility of regenerated silk fibroin film: a medical biomaterial applicable to wound healing. J Zhejiang University Sci B. 2010:11(1):10-16.