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RECENT DEVELOPMENTS IN REGENERATED SILK FIBROIN FIBERS

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ABSTRACT
Silk fiber has gained its importance in the field of tissue engineering due to its excellent mechanical and biocompatible properties. The properties of silk fiber can be engineered for its suitable use in tissue engineering by its regeneration in the form of films or fibers. This review paper summarizes current research related to regeneration techniques for silk fiber preparation, characteristics of regenerated silk fibers and their potential use in the field of biomedical. The regenerated silk fibers are produced by wet-spinning and electrospinning techniques. The effect of process parameters on structure and properties of regenerated silk fibre have been highlighted in this paper. The regenerated silk fibroin fibres find its application mainly in the area of tissue engineering as scaffolds. These scaffolds can be used to support cells for the development of bone, cartilage, vascular grafts and skin tissues.

Keywords: *Regenerated Silk Fiber, Wet-spinning and Electrospinning*

1. INTRODUCTION

Natural silk fiber is obtained from wide variety of insects during their life cycle. Major insects which produce silk fiber are *Bombyx mor*i, *Antheraea mylitta*, *Antheraea assama* and *Antheraea ricini*. The varieties of silk fiber produce by these insects are known as mulberry, tasar, muga and eri respectively. The tasar, eri and muga varieties of silk are also known as non-mulberry silk. [Charu and David (2007)] A single cocoon may provide over 1000 m of fiber by a simple process known as reeling that essentially consists of immersing the cocoon in boiling water. The silk fibers obtained from silkworm have two major protein constituents. Core is made of fibroin protein which is surrounded by outer sericin protein. The core fibroin is made up of two chains known as heavy chain and light chain, mainly constitute of alanine, tyrosine and glycine. Sericin is a globular protein, soluble in hot water, mainly constitute of the amino acids serine, alanine and glycine. Table 1 shows the composition of sericin and fibroin present in different variety of silk. [Mishra (2005), Sen and Babu (2004a)] The composition of amino acids in different variety of silk fibroin is present in Table 2. [Mishra (2005), Sen and Babu (2004b)] The polymer chains of silk fibroin form β-sheet structure. Silk fiber is highly crystalline in nature. The mechanical strength of silk fiber is in the range of 1.9-5.2 g/den.

Table 1. Comparison of silk proteins depending on the origin

	Silkworm Type			
Protein	*Bombyx mori* (Mulberry worm silk)	*Antheraea pernyi* (Tussah or wild silk)	*Antheraea assamensis* (muga silk)	*Philosamia ricini* (eri silk)
Fibroin	70-80%	80-90%	80-90%	80-90%
Sericin	20-30%	8-10%	8-10%	4-5%
Others	2-3%	3-5%	3-5%	3-5%

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Table 2. Chemical composition of different mulberry and non-mulberry silk fibers

Amino Acids	*B. mori*	*A. mylitta*	*A. pernyi*	*P. ricini*
Alanine	29.4	34.1	34.7	36.3
Tyrosine	5.2	6.8	5.1	5.8
Glycine	44.6	27.7	28.4	29.4
Serine	12.1	9.9	9.1	8.9
Aspartic Acid	1.3	6.1	5.0	3.9
Arginine	0.5	2.4	5.0	4.1
Glutamic Acid	1.3	1.3	1.4	1.3
Histidine	0.1	0.8	0.7	0.8

The use of silk fiber in the field of medical is known from centuries as suture. [Lee *et al.*2004] The range of applications of silk fiber is expanding day by day in the field of medical as biomaterials due to its thermal stability, biodegradability and biocompatibility. Silk proteins need to be engineered into useful forms for proper applications in the field of biomedical such as suture, biosensors, scaffold for tissue engineering and wound dressing materials. The properties of silk fiber can be tailor-made for its suitable use in medical textiles either by surface modification or by its regeneration in the form of films or fibers. The regeneration of silk fibers by wet-spinning and electrospinning process have various capabilities, such as controlling the structure and properties of silk fiber, allowing silk fiber to be blended with other polymers, immobilizing the biological materials etc. The regeneration of silk fibers is carried out in three steps, namely degumming, fibroin solution preparation and fiber spinning. Fig. 1 shows the line diagram of the techniques involved in the regeneration process of silk in the form of fibers and films.

The sericin protein present on the outer surface of fiber creates problem in regeneration process. The presence of sericin affects the solubility of fibroin in various solvent for dope preparation. Therefore, it should be completely removed from fibroin surface. The process of removal of sericin is known as degumming. The sericin is removed from the surface of fibroin by boiling the silk cocoon in 0.02 M sodium carbonate solution for 30-40 min. [Min *et al.*(2009)] Another method to remove the sericin from the surface of fibroin is to pressure boil via autoclave. The complete removal of sericin can be achieved by boiling the cocoon at 130°C for 1 hour. [Gulrajani *et al.* (2009)]

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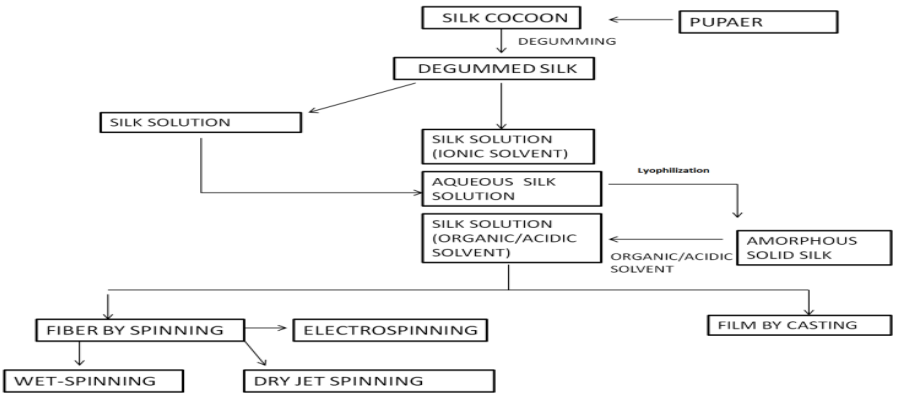

Fig. 1 Line diagram of different methods of silk regeneration

1.1. Preparation of fibroin solution

Silk fibroin can be dissolved in wide range of protic and non-protic solvents. Major solvents which are used for regeneration process are summarized in Table 3. The solubilisation of silk in $Ca(NO_3)_2$-MeOH-H_2O and LiBr–EtOH takes only 10 min while Hexafluoro-isopropanol (HFIP) and hexafluoroacetone take 24 and 2 hours respectively. The $Ca(NO_3)_2$- MeOH-H_2O and LiBr-EtOH-H_2O have strongest solvation for the silk fibroin chains, with almost constant viscosity, while LiBr-H_2O (9.5M LiBr aqueous solution) have the weakest solvation with similar effects on the fibroin molecules to pure water. Recently silk fibers have shown its solubility in eco-friendly solvent N-methyl morpholine-N oxide. The molecular weight of regenerated fibroin in N-methyl morpholine N-oxide is 190 kDa. [Chen *et al*. (2001), Freddi *et al*. (1999), Yao *et al*. (2002)]

Table 3. Solubility of silk fibroin in different types of solvents

Non Protic Solvents	Molar Ratio	Time for solubilisation	References
$Ca(NO_3)_2$-MeOH-H_2O	75:25	10 min	[Chen *et al*. (2001)]
LiBr-EtOH- H_2O	45:44:11	10 min	[Chen *et al*. (2001)]
LiBr-EtOH	40:60	30 min	[Chen *et al*. (2001)]
$CaCl_2$-EtOH- H_2O	1:2:8	10 min	[Chen *et al*. (2001)]
LiBr-H_2O	9.5 M	30 min	[Chen *et al*. (2001)]
N-methyl morpholine *N*-oxide (NMMO)	Absolute	10 min	[Freddi *et al*. (1999]
HFIP	Absolute	24 hr	[Yao *et al*. (2002)]
Hexafluoroacetone Hydrate	Absolute	2 hr	[Yao *et al*. (2002)]

1.2. Rheology of fibroin solution

Viscoelasticity of a polymer solution strongly affects its spinnability, which is related to the flow behaviour of the polymer solution during the fiber spinning process. The rheology of the polymer solution depends on it concentration and its interaction with the solvent. The rheological properties of silk dope solutions are studied by several researchers. [Freddi *et al*. (1999), Yao *et al*. (2002)]

Um *et al.* (2004) have studied the rheological behaviour of silk fibroin solution prepared in 98% formic acid and observed that the silk dope solution showed shear thinning behaviour. The shear thinning behaviour of a polymer solution is characteristic to the molecular entanglement. The entanglement of silk fibroin molecules induced by shear force may occur due to the hydrophobic interaction between molecules or hydrogen bonds between polar groups. Without any external shear force, silk fiber exists as entangled with each other. When the shear rate increases, some portion of entanglement destroyed, resulting in a decrease of shear viscosity. Another important factor in the rheological properties of a dope solution for fiber spinning is the stability of the solution during storage. Viscosity of dope solution can increase or decrease due to the gelation or molecular degradation, respectively. It is observed that the shear viscosity of silk fibroin solution in formic acid remain

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constant for 5 days. The maximum viscosity of dope solution permitted for wet-spinning is 44 Pascal second at 75 °C.

1.3. Techniques for Preparation of Regenerated Fibre

The regenerated silk fibers can be produced by wet-spinning [Um *et al.* (2004), Yazawa *et al.* (1960), Ishizaka *et al.* (1989), Ha *et al.*(2005), Rigueiro *et al.*(2009), Marsano *et al.*(2005)] dry spinning [Sun *et al.*(2012)] and electrospinning [Wnek *et al.*(2003), Fang *et al.*(1997), Son *et al.*(2004), Wang *et al.*(2006), Chen *et al.*(2006), Putthanarat *et al.*(2006), Altman *et al.*(2003), Zarkoob *et al.*(1998a), Zarkoob *et al.*(1998b), Zarkoob *et al.*(2000), Sukigara *et al.*(2003), Ayutsede *et al.*(2005)] techniques. The wet and dry spinning process generates micro diameter fibers. The electrospinning technique produces the nano to submicron diameter fibers.

2. WET-SPINNING

In wet-spinning process, the polymer is dissolved in solvent which is known as dope solution. The polymer dope solution is extruded through spinneret which is immersed in a non-solvent. The polymer in contact with non-solvent gets solidified in the form of filament which then further stretch through subsequent rollers.

Yazawa *et al.* (1960) prepared a concentrated aqueous silk fiber dope solution which was then spun into a coagulation bath of saturated ammonium sulphate solution. Ishizaka *et al.* (1989) used orthophosphoric acid (H_3PO_4) as a solvent for the preparation of silk fiber dope solution, which is spun into ammonium sulphate and sodium sulphate for the preparation of regenerated silk fiber. However, these spinning systems show a serious problem with the degradation of the silk fiber molecules in a phosphoric acid solution. Since then several researchers have prepared regenerated silk fiber using various solvent. The process parameters for regenerated fiber through wet-spinning are summarized in Table 4.

Table 4. Process parameters for wet-spinning of regenerated silk

Solvent	Non-Solvent (Coagulant)	Concentration w /v (%)	Extrusion Speed (ml/min)	Draw ratio	References
Formic Acid	Methanol	13	2.0	3	[Ha *et al.*(2005)]
TFA	Methanol	13	1.5	3	[Ha *et al.*(2005)]
Formic Acid	Methanol	19	0.5	0.64, 1, 2, 3, 4 and 5	[Um *et al.* (2004)]
NMMO	Ethanol	13	8	2	[Rigueiro *et al.*(2009)]
NMMO-H_2O (50/50) w/w	Ethanol	13	4m/min	2.7	[Marsano *et al.*(2005)]
HFA.3H_2O	Methanol	10	-	3	[Yao *et al.* (2002)]

The wet-spinning of silk fiber is carried out by using syringe and syringe pump by extruding the silk dope solution into non-solvent coagulation bath. The complete coagulation of silk fiber takes place from 24 hour to 7 days, depending on solvent/non-solvent system. The process of coagulation of regenerated silk fiber is very slow. The silk fiber can be drawn up to maximum 5 draw ratio. The drawing of regenerated silk fiber can be carried out in wet and hot condition. [Um *et al.* (2004)] Recently, Ha *et al.* (2005) prepared silk fibroin dope solution in formic acid and TFA (13% w/v each) and extrude it in methanol coagulant. The complete regeneration of silk fiber is achieved in 4 hour. Rigueiro *et al.* (2009) spun regenerated silk fiber using eco-friendly solvent NMMO with a dry-jet-wet spinning line. Silk solution is spun at 90°C with 10 mm air gap and coagulated in ethanol at room temperature. Yao *et al.* (2002) reported artificial spinning of *Bombyx mori* silk fibroin with HFA-Hydrated solvent and methanol as non solvent.

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2.1. Characterization of Wet Spun Fibre

2.1.1. Morphological Properties of Wet Spun Fibers

Morphology of regenerated silk fiber is studied with the help of scanning electron microscopy (SEM) [Marsano *et al.* (2005), Sun *et al.* (2012)] and atomic force microscopy (AFM) techniques. [Tao *et al.* (2007)] Regenerated silk fibers with different morphology can be obtained by changing the process parameters such as concentration of coagulation bath, take up rate and subsequent post-spinning draw. The regenerated fibers displayed a fairly regular circular shape with diameter ranging from 133 to 19 μm depending on the spinning parameters. Generally undrawn silk fibers show the largest diameter, which has drastically decreased as soon as drawing applied on fiber.

Marsano *et al.* (2005) have studied surface morphology of wet spun silk fibre by scanning electron microscopy. The surface of undrawn silk fiber shows ridges along the fiber axis, attributed to protein aggregates due to partial stretching by passing through spinning die and/or to protein masses rapidly coagulated when expose to the non-solvent in the coagulation bath. These ridges completely disappear after drawing. [Sun *et al.* (2012)] The characterization of fiber surface by AFM reveals two different microstructures at the nanometer level that differ in the distribution of sizes and in the orientation of the Nano-globules. [Tao *et al.* (2007)]

2.1.2. Structural Properties of Regenerated Silk Fibers

The structural properties of regenerated silk fiber can be studied with X-ray diffraction, fourier transform infrared spectroscopy, Raman spectroscopy and circular dichroism techniques. The regenerated silk filament shows characteristic β-sheet crystalline spacing of 10.1, 4.5 and 3.8 A°, at 2θ = 8.7°, 19.6° and 23.3°, respectively. Um *et al.* (2004) have studied effect of draw ratio on the crystallinity of regenerated fibre. The drawing of regenerated silk filament does not bring about any further crystallization. Silk fibroin filament is crystallized simultaneously by methanol during coagulation process. For this reason, crystallization of the coagulated silk fibroin filament will be difficult due to the suppression of a pre-crystallized region, which leads to no change in crystallinity.

In Raman spectra of regenerated silk fibroin solution obtained by dissolving silk fibroin fibers by lithium thiocyanate and melted $Ca(NO_3)_2$, amide I band shifts to 1650 cm^{-1}, several bands at 1231, 1253, 1262 and 1267 cm^{-1} appear in amide III area and $\nu_{c\text{-}c}$ skeletal stretching band appears at 1102 or 1101 cm^{-1}. These bands are attributed to β-helix and/or random coil conformation, indicating that the molecular conformation of silk fibroin changes from β-sheet to β-helix and/or random coil. [Tao *et al.* (2007)]

FTIR spectra of regenerated silk fibroin films show both random coil and/or β-sheet structure depends on the conformation of silk fibroin, which depends on types of regeneration solvent. [Um *et al.* (2004), Um *et al.* (2001)]

The molecular conformations of regenerated silk fibroin in aqueous solution by circular dichroism shows a strong negative peaks at around 207 and 221 nm, weak peak at around 215 nm, and positive peaks at around 190-198 nm. The appearance of such peaks shows that the regenerated silk fibroin contains mixture of β-helix and random coil structure. [Tao *et al.* (2007), Um *et al.* (2001)]

2.1.3. Thermal Properties of Regenerated Silk

The thermal behaviour of regenerated silk fibroin was investigated by means of differential scanning calorimetry (DSC) and thermogravimetric (TGA) measurements. The DSC curve displayed two broad endotherms, one at low temperature, due to loss of moisture, another at high temperature (endothermic peak at 294°C), attributed to thermal decomposition of silk. Well oriented silk fibers usually exhibit a decomposition peak above 300°C. Unoriented silk materials with β-sheet crystalline structure usually decompose in the temperature ranges of 290-295°C. The decomposition of amorphous silk fibroin occurs at lower temperature at 290°C. [Freddi *et al.* (1999)] The maximum decomposition temperature of unoriented filament occurred at 314°C and shifted to slightly higher temperature with increase in draw ratio. [Um *et al.* (2004)]

3. ELECTROSPINNING

Electrospinning is a type of dry spinning process for production of nano to submicron diameter fibers. First patent for fabrication of various nonwoven materials by electrospinning machine was filed by Formhals in 1934. [Formhals (1934)] A typical electrospinning setup consists of three components; a high voltage supplier, a capillary needle or pipette and a grounded collector. The electrospinning technique involves the generation of a strong electric field between a polymer solution contained in a reservoir such as a glass syringe with a capillary

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tip or needle, and a metallic collection plate. At the critical voltage the electric force from the applied field become higher than the surface tension of the droplet i.e. as the charge overcomes the surface tension of the deformed drop of suspended polymer solution formed on the capillary tip or needle results in the ejection of charged jet of the solution in the direction of the applied field. Under electro-hydrodynamic forces the diameter of electrically charged jet decreases and this jet undergoes a series of several electrically induced bending instabilities during passage to the collection plate, which results in extensive stretching. [Jin *et al.* (2002)] During stretching process rapid evaporation of the solvent take place, which results in the reduction in the diameter of the jet. After stretching and drying, these fibers collect randomly or in an aligned manner on the surface of collecting plate. The diameter of electrospun nano-fiber can be controlled by changing the processing parameters such as concentration of solution, viscosity and electric field; type of solvent employed, distance from tip-to-collection plate, flow rate, [Shin *et al.* (2001)] diameter and angle of the spinneret. [Deitzel *et al.* (2001)]

Recently, regenerated silk fibroin fiber with submicron diameter was prepared through electrospinning process. [Ohgo *et al.* (2003), Wnek *et al.* (2003), Fang *et al.* (1997), Son *et al.* (2004), Wang *et al*. (2006), Chen *et al*. (2006), Putthanarat *et al*. (2006), Altman *et al*. (2003) The electrospinning of *B. mori* cocoon silk and *Nephila clavipes* dragline silks were first reported by Putthanarat *et al*. (2006), Altman *et al*. (2003) and Zarkoob *et al.* (1998). They used solution of 0.23–1.2 wt% of silk fibroin and spidroin in hexafluoro-2-propanol. [Zarkoob *et al.* (1998b), Zarkoob *et al.* (2000), Sukigara *et al.* (2003)]

3.1. Process Parameters for Electrospinning of Silk

There are many parameters that affect the electrospinning of a silk fibroin solution and their blends such as the interaction between polymer and solution, chemistry, molecular weight, molecular weight distribution of the polymer and the rheological behaviour of the solution (concentration, viscosity, elasticity, conductivity and surface tension). The parameters such as hydrostatic pressure in syringe and needle, applied voltage, distance between tip and collector and flow rate of solution have great influence on processing of silk by electrospinning. The process parameters which affect the formation of electrospun fiber are summarized in Table 5.

Table 5. Process parameters for electrospinning of silk fibroin

Type of Silk	Fibroin Concentration (%)	Solvent	Electric Field (KV/cm)	Spinning Distance (cm)	Av. Fiber Diameter (nm)	References
Bombyx mori	8	Formic Acid	4	7	26.3	[Sukigara *et al.*(2003)]
Bombyx mori	9	Formic Acid	3	10	52.2	[Ayutsede *et al.*(2005)]
N. Clvipes (Dragline)	0.74	Hexafluoro-2-propanol	24-30 (KV)	15	100	[Zarkoob *et al.* (2004)]
Bombyx mori	8,9,10,11	Formic Acid	12 (KV)	10	205,235, 305,320	[Lee *et al.* (2005)]
Tussah	10	HFIP	12 (KV)	12	---	[Zhang *et al.* (2010)]
Bombyx mori	12	Formic acid	2 to 4	7	70 to 45	[Sukigara *et al*. (2004)]
Bombyx mori	38	Aqueous	20 (KV)	20	2113	[Zhu *et al.* (2007)]

Many researchers have investigated that there is a relationship between polymer concentration and fiber diameter. [Sukigara *et al.* (2003), Ayutsede *et al.* (2005)] It is studied that increasing the concentration of silk solution results in increased fiber diameter and decreased bead formation. If the solution concentration of *Bombyx mori* silk formic acid is too low (5%) then the fiber break into microsize droplets before reaching the collector so fiber will not be formed which result in phenomena of electrospray rather than electrospinning. [Ayutsede *et al.* (2005)] The phenomenon of electrospray is also observed when a solution of low molecular weight polymer is used. It is also noted that if the concentration of silk solution is too high, it will be difficult for the polymer solution to flow through the capillary due to high viscosity; therefore no fiber will be formed. [Zhang *et al* (2009)] The type of solvent also affects the electrospinnability of silk fiber. The effect of solvent on the diameter of electrospun *Bombyx mori* silk fiber has also been studied by Jeong *et al.* (2007). In this study it is found that mean diameter of electrospun nano-fibers from silk formic acid solution are smaller (80 nm) than

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those from HFIP silk solution (380 nm). This is only due to faster evaporation of HFIP than formic acid, which led to the formation of thicker fibers with less elongation. It is interesting to note that from 17% aqueous *Bombyx mori* silk solution electrospun fiber is not formed, while at 28% (w/v) silk fiber with diameter from 400-800 nm is observed, with circular cross-sections and smooth exterior surfaces. At 39% (w/v) aqueous silk fiber solution an un-even and ribbon shaped silk fiber formed possibly due to slow rate of evaporation from fiber surface before the fiber reach the collector. Zhu *et al.* (2007) found that from 30% (w/v) aqueous silk solution, electrospun fiber formed with diameter of 1749 nm but as the concentration increase from 33 to 38% the diameter of electrospun fibers reaches to 2275 and 2113 nm.

The pH and concentration have a remarkable influence on the properties of regenerated silk fibroin aqueous solutions. [Zhu *et al.* (2008)] The lowering of pH could induce gel formation and decrease the electrospinnability of regenerated silk fibroin aqueous solutions. The uniform cylindrical fibers with a smaller average diameter of 265 nm obtained by using the solution at a pH of 4.8. At the pH 6.0 and concentration of 33 wt%, a uniform cylindrical fiber with an average diameter of 718 nm is obtained.

Applied electric potential is the one of most important process parameter of electrospinning which has great influence on fiber diameter, jet formation and bead formation. Sukigara *et al.* (2003) demonstrated that at 3 KV/cm electric potential, fiber did not formed from *Bombyx mori* silk-formic acid solution but when 4 KV/cm electric filed applied then fiber jet induced and causes the formation of fiber with diameter of 26.3 nm. This suggests that a sufficient electrostatic force can overcome solution surface tension and further extract silk fibroin chains. [Meechaisue *et al.* (2007)]

The flow rate also has great influence on the electrospinnability of silk fiber. The feed rate controls the volume of solution suspended at tip of the spinnerette that maintain the shape of Taylor cone. The maintenance of Taylor cone is very important for jet formation. Between 10 KV and 20 KV formation of stable jet is observed. The fiber diameter is narrowest at 10 KV (lowest electric field) for different concentration. [Amiraliyan *et al.* (2009)]

3.2. Post Treatment of Electrospun Fibers

Electrospun silk fiber generally have random coil, α-helix (Silk I) and β-sheet conformation (Silk II). The conformation transition from random coil and α-helix to β-sheet (crystallization) can be induced by chemical treatment. The chemical treatment involves use of low dielectric constant organic solvents mainly methanol and ethanol for effective crystallization of silk nano-fibers. During crystallization alcohols attract the water from silk fibroin molecules due to their polar character and this promotes the aggregation of hydrophobic amino acids such as alanine and glycine. The crystallinity of methanol treated electrospun silk fiber is 38.2 while ethanol treated electrospun silk fiber is 43.4. [Amiraliyan *et al.* (2009)] Water is also used to anneal the silk fibers. It is used to produce water stable silk fibroin matrix. [Jin *et al.* (2005), Asakura *et al.* (1992)]

3.3. Performance Properties of Electrospun Mat

The mechanical properties of the electrospun mats were found to be dependent on fiber diameter. The mats containing fibers have higher tensile strength but lower breaking strain due to large number of fiber-to-fiber contacts due to nanoscale fiber diameter. Tensile strengths of electrospun mats enhance after methanol and ethanol treatment at the expenses of their breaking strain. [Ayutsede et al. (2005), Asakura *et al.* (1992)]

Suan Fan *et al.* (2013) found that the mats that are mechanically drawn at 1.4× draw ratio and 0.1 mm/s draw rate in 90% ethanol aqueous solution and then immersed in the same solution for 30 min, have showed higher breaking strength up to 8.6 MPa and breaking energy 172.2 J/kg, respectively. However, those of the as-spun mats are only 1.8 MPa and 93.2 J/kg, respectively.

3.4. Morphology of Electrospun Nano-fibers

The morphology of the electrospun fibers changed gradually from beaded fibers to circular cross section fibers, and then to ribbon-like fibers with the increase of the concentration of spinning dope. When the concentration of the solutions are 11 wt% and 17 wt%, the corresponding thickness of the electrospun fibers were about 0.21±0.03 μm and 0.49±0.06 μm, respectively. It could be seen that there was a significant decrease in the diameter of the regenerated silk fiber from 0.49±0.06 μm to 0.12±0.02 μm when the electrical conductivity of the solution increased from 1.000 mS/cm to 7.400 mS/cm. [Cao *et al.* (2009)]

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3.5. Structural Analysis by Wide Angle X-Ray Diffraction (WAXD):

The electrospun fiber is characterized by diffraction peaks at 13.7, 16.6, 18.3, 25.4 and 28.68, corresponding to silk (I) and silk (II) crystalline d-spacing of 6.5, 5.3 (II), 4.8 (II), 3.5 (I) and 3.1 (I) A° , respectively. These diffraction peaks indicate preferred orientation of the molecular axis of the crystals along the fiber axis. The electro-spun fiber is comprised of mixtures of random (silk (I)) and β-sheet (silk (II)) crystals. The crystallinity of electrospun fibers is obtained 39%. [Ayutsede *et al*. (2005)]

4. APPLICATIONS OF REGENERATED SILK FIBROIN FIBERS

The regenerated silk fibroin fibres have application mainly in the area of tissue engineering as scaffolds. These scaffolds can be used to support the cells for development of bone [Zhang *et al*. (2010), Park *et al*. (2010), Correia *et al*. (2012), Li *et al*. (2006)], cartilage [Morri *et al*. (2003), Wang *et al*. (2005), Wang *et al*. (2006)], vascular grafts [Wissin *et al*. (2000), Motlagh *et al*. (2006), Marelli *et al*. (2010), Zhang *et al*. (2010), Lovett *et al*. (2007), Liu *et al*. (2011)] and skin tissues. [Min et al. (2004)]

Park *et al.* (2010) have fabricated three-dimensional electrospun silk fibroin scaffolds with controllable pore size to evaluate suitability of porous electrospun silk fibroin scaffolds for bone regeneration via *in vitro* and *in vivo* studies. Porous electrospun silk fibroin scaffolds support significantly higher proliferation and alkaline phosphatase activity of osteoblasts than PLA *in vitro* ($p<0.05$). [Park *et al*. (2010)] Porous electrospun silk fibroin scaffolds with control pore size when seeded with human adipose derived stem cells (hASCs) in osteogenic medium shows bone protein production. [Correia *et al*. (2012)] The coexistence of bone morphogenetic protein-2 (BMP-2) and nanoparticles of hydroxyapatite (nHAP) in the electrospun silk fibroin fiber show highest calcium deposition. [Li *et al*. (2006)] Mesenchymal stem cells (MSCs) and chondrocytes are two important cell sources for cartilage tissue engineering. It is observed that the MSCs cells are attached more efficiently to aqueous-derived silk fibroin scaffolds. [Morri *et al*. (2003), Wang *et al*. (2005), Wang *et al*. (2006)]

Silk fibroin is a suitable material for vascular tissue engineering due to its ability to support the attachment, proliferation and differentiation of vascular tissues and resist shear stress and pressure from simulated blood flow. [Wissin *et al*. (2000), Motlagh *et al*. (2006)] The tubular silk scaffolds electrospun out from formic acid can resists up to 575 mm Hg, which is more than four time the upper physiological pressure of 120 mm Hg & twice that of pathological pressure of 180-220 mm Hg. The *in vitro* tests showed a good cytocompatibility of the electrospun silk fibre tubes. [Marelli *et al*. (2010)] For vascular applications aqueous silk fibroin solution with addition of poly (ethylene oxide) (PEO) is preferred. [Zhang *et al*. (2010)] Lovett *et al*. (2007) prepared micro-tubes by dipping straight lengths of stainless steel wire into aqueous silk fibroin, with the addition of PEO that control micro-tube porosity. Low porosity micro-tubes demonstrated superior mechanical properties in terms of higher burst pressures, but displayed poor protein permeability; whereas higher porosity tubes had lower burst strengths but increased permeability and enhanced protein transport. [Liu *et al*. (2011), Yang *et al*. (2007)]

The damage of skin due to burns, wounds or disease may results in significant disability or even death. The skin grafting is a challenging task for surgeons because of transplant limitations. Silk fibroin nanofibre nonwoven matrix gives support to seed the normal human keratinocytes. [Min *et al.* (2004)]

5. CONCLUSION

The research on regenerated silk fiber is mainly restricted to *Bombyx mori* or mulberry silk. A few studies are carried out on regeneration of non-mulberry or wild silk. However they are also potential candidates for biomedical applications. The rheological properties of silk fibroin solution mainly focus on shear thinning behaviour and solution stability. Limited studies have been reported on the effect of temperature on flow behaviour, molecular weight and molecular weight distribution as it affects the spinnability of the fiber. The process of wet-spinning is mainly carried out with syringe and syringe pump. Few studies on lab model wet-spinning machine are reported. To develop regenerated fiber at commercial level it is highly needed to carry out the experiments at lab model pilot scale machine. Enough studies have been done to elucidate the factors that have great influence on electrospinning, their relationships and resulting fibers characteristics. However, it is still difficult to predict the outcome from an electrospinning process due to complex and interrelated effects from various parameters. A unique challenge for silk biomaterials is to maintain batch-to-batch consistency due to variations of composition as well as material processing. Therefore, efforts are needed to explore potential of regenerated silk biopolymer.

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