

## **Silk in Biomedical Engineering: A Review**

Zubir, N.<sup>1</sup>, Pushpanathan, K.<sup>2</sup>

<sup>1</sup> Department of Biology, Centre for Foundation Studies (CFS), International Islamic University Malaysia (IIUM), JalanUniversiti, 46350 Petaling Jaya, Selangor

<sup>2</sup>Department of Biomedical Engineering, Faculty of Engineering, University of Malaya, 50603 Kuala Lumpur, Malaysia

---

**Abstract:**-The astounding biomaterial features of silk for example its high mechanical strength, biocompatibility and degradability have led to plethoric study on the silk properties. Tissue engineering and regenerative medicine are the fields that have been widely explored using silk scaffolds due to their mechanical stability. The mechanical stability of the scaffold is vital for the cells to adhere, expand, divide, proliferate and differentiate. Various types of connective tissues for examples tendon, ligament, bone and cartilage have been used. Apart from that, the scaffold should also display remarkable biocompatibility feature, should be porous with high surface area, should match the target tissue and reproducible. Another important aspect of the scaffold that needs significant consideration is the usage of nanoscale structure for the cells to be able to recognize and elicit response. Hence, nanofibres is the most suitable format of the artificial matrices to be considered in the tissue engineering technique. Inexpensive and simple approach pertaining to nanofibres approach is electrospinning method which capable to be scaled up and to produce 3 dimensional, porous structure with high surface area. Vast studies on the tissue engineering pertaining to silk and the silk regenerative properties have been associated with the impressive mechanical strength of the silk.

**Keywords:** *biocompatibility, biomaterial, cell growth, sericine, silk fibroin*

---

### **I. INTRODUCTION**

The use of silk in medicine has spanned for centuries as a suture material [1]. In the field of regenerative medicine, silk is used as a scaffold in engineering connective tissues: tendon, ligament, bone and cartilage. Its outstanding biomaterial properties has attracted numerous and extensive research to study its potential use. Silk is tough but light weight and biodegradable. The silk's morphologies and its biocompatibility are much of desire for it elicits less or no immune response in hosts [2].

Silks are produced by silkworms, spiders, fleas, mites and scorpion. These animals use silk for preying, protection and even reproduction [3, 4]. Silks are a protein polymer of large molecular weight, hydrophobic and thermally stable at extreme temperatures. Its properties vary depending on the source, surrounding temperature and the way they are harvested. For example: compared to cocoon silks, immobilized silkworms silks which were artificially extracted are far better. Silks from forced silking technique, compared to silks collected from freely walking spiders are significantly different in their respective properties [3, 5].

Assessment of biodegradability of silk scaffold is of most importance, for silk is highly regarded as non-degradable material. Silk fibroin (SF, extracted from B. Mori) takes over 60 days for in vivo degradation. For a material to be absorbable, it needs to lose most of its tensile strength within 60 days in vivo. However, dragline spider silk with its rapid degradation when implanted in the skin could be possibly to be used as a scaffold [6, 7, 8]. Silk protein occurs in crystalline  $\beta$  sheets structure, composed of mainly fibroin and sericin. The repetitive sequence in fibroin polymer could contribute to the structure of cocoon, nest and web formation while the crystalline  $\beta$  sheets arrangement plays a huge role on the degradability of silk [9, 10, 11, 12].

Research has been carried out using silk fibroin in investigating the adhesion, proliferation and differentiation of stem cell in vivo, in addition to the in vivo tissue repair [13]. Controlling variables that affect spider silk assembly and processing in vitro as well in vivo, such as solvent, pH, water, salt and protein concentration has proved to be of many difficulties. However, focusing on degradability assessment, in vitro system setting provide simpler and cheaper alternative [5].

At the molecular level, tissue regeneration is highly dependent on the extracellular matrix (ECM). The cells need a support platform for proliferation and differentiation into tissues. Therefore for the scaffold to mimic the ECM, it should have great biocompatibility, porous to allow oxygen and nutrition diffusion, with large surface area, match the target tissue and reproducible [14].

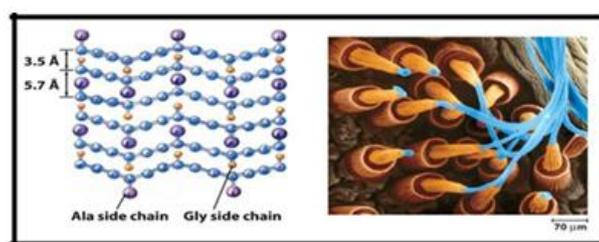
For cells to be able to recognize and elicit response, the scaffold needs to be in nanoscale, therefore nanofibers is the best setup to be used tissue engineering technique. Electrospinning method provides simple and cheap approach in nanoscale and can be scaled up. Different variables such as substrates, solutions and

environment can also be adjusted [15, 16, 17, 18, 19]. Isolated mesenchymal stem cells (MSCs) from adult bone marrow was used in in vitro replication of cartilage with highly porous 3D silk scaffold. The MSCs was found out to be attached, proliferated and differentiated in the silk scaffold [20]. A study using electrospun silk matrices scaffold and human bone marrow stromal cell (BMCs) also found out that the BMCs adhere, differentiate and proliferate on the silk matrices [21]. In another study, human keratinocytes and fibroblast cultures was observed to adhere and spreading type I collagen triggered by electrospun SF nanofibers which give large surface area for cell attachment within the 3D feature [22].

Further discoveries on how connective tissues regenerate could be applied in peripheral nerve regeneration by using silk as the scaffold. Sericin which is a major component of silk was tested in vivo for peripheral nerve regeneration, with the same mechanical strength of porous conduit found in peripheral nerve tissue. Investigation of rat sciatic nerve repair was conducted, using fabricated sericine/silicone double conduit to achieve maximum functional recovery as the single conduit could only achieve marginal recovery [23].

## II. PROPERTIES OF SILK

The fibrous silk protein is mainly composed of fibroin and sericin. By referring to Figure 1, Silk Fibroin (SF) consists of layers of antiparallel  $\beta$  crystalline or pleated structures which rich in amino acid Alanine (Ala) and Glycine (Gly). The  $\beta$  crystalline structure contributes to its high mechanical strength and had much influence on the degradability property of silk as the biomaterial [9, 10, 11, 12].



**Figure 1. Silk Fibroin (SF) [24]**

Very large molecular weight, hydrophobic, thermally stable at very high or low temperature are some of the properties of silk which depend on the sources, conditions for instance climate and ambience apart from how they are produced. Silks which are derived from immobilized silkworms are more superior to natural cocoon silks. Silks obtained from the forced silking have different features and properties when compared with the silks obtained from freely walking spider [3, 5]. One of the prominent properties that need to be evaluated before using silk as a scaffold is biodegradable. It is referred to an absorbable biomaterial if one loses most of its tensile strength within 60 days in vivo. Silk fibroin (SF), extracted from the silkworm B. Mori takes over 60 days to degrade in vivo and thus it is considered as non-degradable material. Nevertheless, due to very rapid degradability when implanted in the skin, dragline spider silk may face an obstacle if it is used as a scaffold [6, 7, 8]. Silk have often been the focus of the medical field due to its strength and biocompatibility in human. Using silk in the field of tissue engineering would be biomimicry at its best. By exploiting the non-linear behaviour of the silk, silk can be used in various fields especially in the field of medicine. Silk can be used as drug carriers or even scaffolds. Silk scaffolds are mainly used in bone regeneration owing to the strength of the material while still exhibiting cytocompatibility, low immunogenicity, biodegradability, low toxicity both in vivo and in vitro [25]. The following table (Table 1) shows the comparison between man-made fibres and silk in terms of mechanical properties [26].

**Table 1: Comparison between the mechanical properties of silk and man-made fibres [26]**

Material	Strength, max/GPa	Extensibility, max	Toughness/ MJm <sup>-3</sup>
B. mori silk	0.6	0.18	70
A. diadematus MA silk	1.1	0.27	160
A. diadematus Flag silk	0.5	2.70	150
Nylon	0.95	0.18	80
Kevlar49™	3.6	0.027	50
High-tensile steel	1.5	0.008	6

### III PROTEIN PROPERTIES AND CHARACTERISTICS

During metamorphosis, silkworms produce cocoons which are polyamino acid-based to protect themselves whereas spiders produce spidroins [26]. Both polyamino acid and spidroins are both made up of amino acids [27]. Polyamino acids are synthesised when DNA is used as a blueprint in the mRNA production through a process of transcription. The mRNA is then used as a blueprint by the ribosome for the production of polypeptide which are sequence specific where the process is known as translation. The translation process is basically the translation of information stored in polynucleic acid which is the genetic code into polyamino acid [28]. The primary structure of the material is the sequence of residues in the polypeptide which exhibits different functional groups. These functional groups are grouped into polar, non-polar, aromatic, anionic or cationic [29]. Secondary structures such as  $\alpha$ -helices,  $\beta$ -sheets and  $\gamma$ -turns which are determined by supramolecular interactions - hydrogen bonding between amide bonds or  $\pi$  interactions between aromatics groups [30, 31].

Formation of  $\alpha$ -helices occurs when hydrogen bonds between hydrogen atom attached to the nitrogen atom of an amide and the carbonyl atom of the fourth on the amino-terminal side of the peptide bond causes polymer to coil into an axis. The  $\alpha$ -helix is stimulated by ion pair formation between oppositely charged residues which are 3 to 4 amino acids apart. It is also stimulated by the  $\pi$ -interactions between the aromatic amino acid which are spaced as the former [32]. As for the  $\beta$ -sheets formation, hydrogen bonding between the chains causes the polypeptide chain to take a form of zigzag.  $\beta$ -sheets formation is possible with the amino acids with small chains such as glycine and alanine. The formation of  $\beta$ -sheets would not be possible with amino acids with larger chains [33].

The 3-D arrangement of the polypeptide chains is totally dependent cross-links which are either covalent or non-covalent between the different regions within the chain while the primary structure of polypeptide is responsible for the regions which are either locally disorganised or organised - which is the tertiary structure [34]. Quaternary structure occurs when different polypeptide chains interact with each other. The process 'folding' takes place after polymerisation which is aided by other proteins [35].

### IV TYPES OF SILKS

#### Silkworm silk

The most commonly used silk is from B.mori. The silk extracted from B.mori has a core-shell structure. The core is made up of heavy chain fibroin, light chain fibroin and glycoprotein which are proteins. The heavy chain fibroin or H-chain has a molecular weight of 350kDa. H-chain is hydrophobic and contributes to the crystalline structure of the silk. This H-chain is connected with light chain fibroin (L-chain) through disulphide bond which has a molecular weight of 25kDa. L-chain is more hydrophilic and elastic compared to H-chain. The glycoprotein which is actually known as P25 has a molecular weight of 30kDa. This protein is linked with the other two components of the core through non-covalent hydrophobic interactions. P25 contributes to the

structural strength of silk as a whole. The molar ratio of these three proteins – H-chain: L-chain: P25 is 6:6:1 [36, 37, 38].

### **Spider silk**

Spider silks consists of many silks which are made up of different compositions of proteins and has different mechanical properties from each other.

### **Major Ampullate silk (MA silk)**

This type of silk has a very high tensile strength and has moderate elasticity with molecular weight more than 300kDa. MA silk has a structure similar to silk produced by silkworm. This silk has a diameter between 1 to 20mm which depends entirely on the species of the spiders. Two major protein components of this type of silk are Major Ampullate Spidroins 1 and 2 which mainly consists of glycine, alanine and proline [39, 40].

### **Minor Ampullate silk**

Minor ampullate silk have similar mechanical properties as Major Ampullate silk. It has a molecular weight of more than 250 kDa. The major protein components are Minor ampullate spidroins 1 and 2 which are similar to Major Ampullate silk in terms of composition but with major differences – there are no presence of proline and amount of glutamate is reduced [41,42].

### **Flagelliform silk**

Flagelliform silk are highly elastics and has a molecular weight of 500 kDa. This type of silk is made up of one major protein which contains higher amount of proline and valine and reduced amount of alanine [43].

### **Pyriform silk**

This silk has low amount of small non-polar amino acids and higher amount of polar and charged amino acids – which are significant for cross-linking [44].

### **Aggregate silk**

This type of silk consists of glycoproteins and small hygroscopic peptides. It has small amounts of non-polar amino acids while proline quantity is significantly higher. Cross-linking and hydration of the fibres are mainly contributed by polar and charged amino acids [45].

### **Silk Processing**

Manipulating factors such as solvent, pH, water, concentration of salt or concentration of protein which affects the assembly and processing of spider silk both in vivo and in vitro is one of the obstacles faced by researchers. However, this system provides a much simpler and cheaper conditions in vitro as more emphasis is given towards evaluating the degradability [5]. Tissue regeneration is dependent on extracellular matrix (ECM). Scaffolds are fundamental to tissue regeneration. The constructs act as mechanical support for the cells and allowing the cells to divide, proliferate and differentiate into tissues. Therefore, scaffolds need to mimic natural ECM by having favourable characteristics such as biocompatibility, porosity and reproducibility [14]. Using nanoscale structures in scaffolds would allow the cells to recognize and stimulate response. Therefore, nanotechnology integrated in fabrication of scaffolds would be a great advancement in tissue regeneration. Electrospinning method would be an inexpensive and simple approach in obtaining the nanofibres and has the potential to be scaled up.

Apart from the factors mentioned earlier, parameters such as substrates, solutions and environment can be manipulated [15, 16, 17, 18, 19]. Environmental factors such as pH, water content, concentration of protein, salt, and charge can have an impact on the processing and assembly of spider silk in vivo and in vitro. Interchain interactions for example are increased with enhanced calcium concentration and low water content [46].

Hydrogels which are developed from the recombinant spider silk exhibit high elastic modulus and were discovered to be stable for several weeks. Polymer hydrogel have been researched in tissue engineering for the development of porous yet stable scaffolds. Nanofiber-based hydrogels were produced from engineered and recombinant spider silk proteins. The silk nanofibers are stable semi-flexible polymers which assemble into hydrogel networks.

Native silk fibres can be processed into wire ropes and non-woven silk mats which can be solubilized and regenerated in aqueous solution then further processed into sponges, films, hydrogels, and nanoscale electrospun nonwoven mats. Surface modification of silk fibroin is vital as it can be used to alter cell responses. Processing conditions can affect the degradability of silk fibroin. The field of molecular biology have been

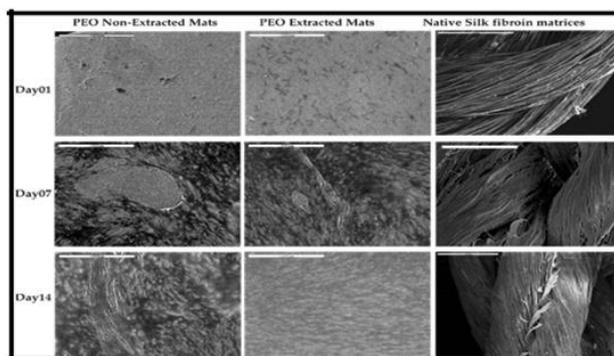
successful in generating spider silk although the quantity of silk produced is not satisfactory. These genetically engineered spider silk proteins which have been formed into films can be chemically modified [47].

## V APPLICATION AND RESEARCH ON SILK: PAST AND PRESENT

Silk, in the form of sutures, has been used for centuries [1]. In the 19th century, Louis Pasteur who has made a significant contribution by saving the silk industry in France from bacterial infection and indirectly triggered the development of silk in the western countries. In 1901, Dr. Ishiwata Shigetane successfully discovered and identified the bacteria strain which was affecting the silkworm. The bacteria strain is *Bacillus thuringiensis*. Only in 19th century, metal wires were replaced with silk for sutures by the surgeons [48].

Numerous research have been conducted on silk due to its superior and remarkable biomaterial properties for example high strength, toughness, lightweight, biocompatibility, and degradability [49]. The main component of the silk which is silk fibroin (SF) has been used to explore the adhesion, proliferation and differentiation of stem cell in vivo apart from tissue repair [13].

The electrospun SF nanofibers demonstrated a high level of surface area with the three dimensional feature for the attachment of cells. The feature was observed to have stimulated the cell adhesion and spreading of type I collagen [22]. In 2004, the potential use of the electrospun silk matrices was investigated using the Human bone marrow stromal cells (BMSCs). The aqueous regenerated silkworm silk solutions was used to prepare the silk matrices [21]. The electrospun silk fibroin-based fibers with average diameter 700-750 nm (Fig 2) has shown to promote the adhesion, spreading and proliferation of BMSCs. The ability of the electrospun silk matrices to support the attachment, spreading and growth of the BMSCs in vitro due to its mechanical strength apart from its biocompatibility and biodegradable properties. (Fig 2)



**Figure 2.** Scanning electron micrographs of BMSCs growing on electrospun mats and native silk fibroin matrices after 1, 7 and 14 days (scale bar: 5mm) [21]

The fabrication of silk fibroin (SF) nanofiber nonwovens for cell culture of normal human keratinocytes and fibroblasts was carried out. They adopted the electrospinning method in their study to explore the cell attachment and spreading. The morphology and microstructure of as-spun and chemically treated SF nanofibers were studied using scanning electron microscopy (SEM) and mercury porosimetry. The cytocompatibility and cell behaviour onto the electrospun SF nanofibers were also studied [22].

In 2005, adult mesenchymal stem cells (MSCs) were isolated from adult whole bone marrow to study on their attachment, spreading and proliferation on the highly porous three dimensional silk scaffold [20]. The study has shown that MSCs attached, proliferated and differentiated in the silk scaffold.

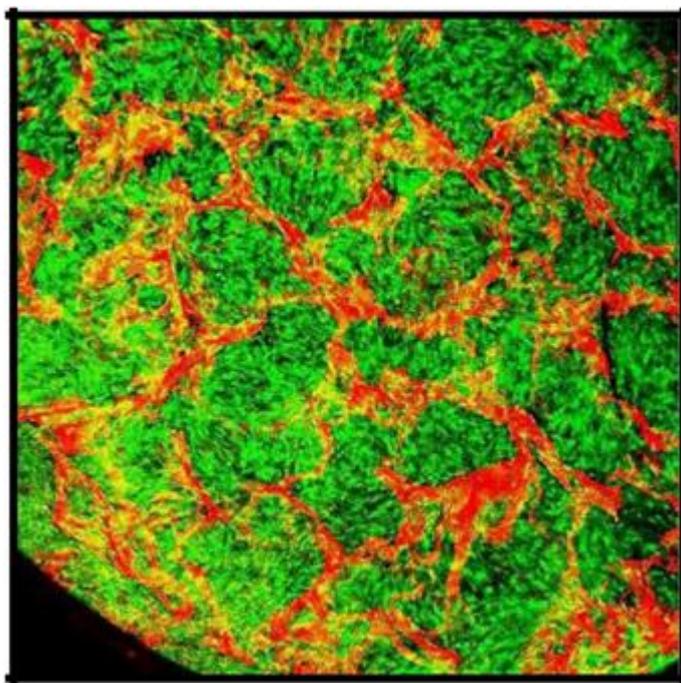
In another study, scaffolds which range from twisted fibre architectures composed of silk fibres to complex three-dimensional braided structures composed of poly (L-lactic acid) fibres were explored. Among the objectives of these tissue-engineered are the application of growth factors to promote ligament tissue regeneration while providing mechanical properties similar to natural ligament constructs apart from applying approaches such as the use of porous scaffolds as well as the use of cells [50].

The limited ability of the self-repair capacity is experienced by adult cartilage tissue, especially in the case of severe damages for examples developmental abnormalities, trauma, or aging-related degeneration. Adult mesenchymal stem cells (MSCs) were observed to have the ability to divide and differentiate into bone, cartilage, and fat cells. The spatial cell arrangement and distribution in the MSCs-silk scaffold constructs mimic those in native articular cartilage tissue after 3 weeks of cultivation [20].

In another study, the electrospinning method was also used to fabricate the silk fibroin fibre scaffolds. Bone morphogenetic protein 2 (BMP-2) and/or nanoparticles of hydroxyapatite (nHAP) were used for the in vitro bone formation from human bone marrow-derived mesenchymal stem cells (hMSCs). It has been

demonstrated that the electrospun silk fibroin-based scaffolds supported hMSC proliferation, growth and differentiation [15]. Films, fibres, nets, meshes, membranes, yarns, and sponges are various formats of silk fibroin (SF) which have been widely explored. They have been observed to support stem cell adhesion, proliferation, and differentiation in vitro apart from to trigger tissue repair in vivo. The silk-based biomaterials have been shown to be promising scaffolds for tissue engineering of bone, ligament, cartilage, stem cells as well as skin. By referring to figure 3, the three-dimensional distribution of human bone marrow derived mesenchymal stem cells was observed in porous aqueous derived silk fibroin scaffolds at week 3 [13].

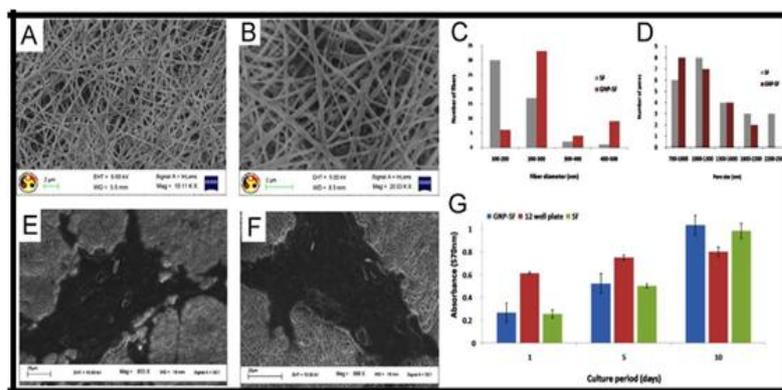
In 2008, increased amount of primineralized silk fibroin using Hydroxyapatite (HAP) and BMP-2 was used [51]. The increased in osteoconductive and biomimetic growth of calcium phosphate were shown on silk fibroin. Enhanced outcome for bone tissue engineering was demonstrated in the study. ‘



**Figure 3.** Cells were stained using 2 mMcalcein AM (green for live cells) and 4 mMethD-1 (red for mostly silk fibroin and also dead cells) (Molecular Probes, Eugene, OR) for 30-45 min at room temperature and evaluated using a Bio-Rad MRC 1024 confocal microscope with Laserssharp 2000 software [13].

The exploration on the nerve regeneration was triggered by the plethoric information and study on the regeneration of connective tissues. Silk has been identified as the potential biomaterial to regenerate damaged peripheral nerves for instance, sensory neuron, motor neuron or sciatic nerves. Another major component of silk, sericin, has been studied in vivo for the peripheral nerve regeneration. The recovery of a rat sciatic nerve was studied using the double conduits, sericine/silicone. It has been observed that the mechanical strength of conduit porous matched the peripheral nerve tissue. The double conduit was developed to attain maximum functional recovery. This is because the single conduit could only reach marginal recovery [52].

The Schwann cells were observed to attach, adhere and proliferate over the surface of electrospun nanofibers (Fig. 4A, B, E and F) [53]. The subsequent increased of absorbance with time over a period of 10 days when compared to a more saturated growth in control group of 12 well tissue culture plate (Fig. 4G) has been demonstrated in the assay of cells growing over the scaffolds. Hence, the increased cellular proliferation over porous nano- scaffolds with larger surface area to volume ratio than conventional tissue culture plates with similar dimensions was exhibited. From this experiment, it can be concluded that the silk has the potential and can be exploited to promote nerve regeneration, particularly peripheral nerve [53].



**Figure 4.** Architecture of nanofibers and culture of Schwann cells over nanofibrous scaffolds. (A) SF nanofibrous scaffold and (B) nanocomposite (GNP-SF) nanofibrous scaffold. (E) SF nanofibrous scaffold after 5 days of culturing Schwann cells and (F) GNP-SF nanocomposite nanofibrous scaffold after 5 days of culturing Schwann cells (G) [53]

Composite silk fibres with improved overall mechanical properties can be manufactured through the creation of transgenic silkworms which encoded chimeric silkworm or spider silk proteins. The piggyBac vectors were used in the process of forming the transgenic silkworms [54].

In a study of a bilayered vascular graft, the graft which is developed from silk fibroin (SF) proteins demonstrated improved mechanical strength, mechanical compliance, and suture retention strength as well as burst pressure. The bilayered graft comprises of an inner layer of silk fibre tube with heparin and the external layer consists of highly porous silk fibre. The result showed that apart from the blood compatibility to the silk proteins based graft, the heparin release was able to be sustained for at least a month. The highly porous structure exhibited by the external layer of the graft was prepared through lyophilization technique. The macropore walls with nanofiber structures were highly porous and mimic to extracellular matrix. This provides remarkable property for the cell division, proliferation and growth. Another properties for example good cytocompatibility and hemocompatibility were also shown by the in vitro studies. [55]. A study on the layered structures of the composite films of graphene oxide (GO) sheets and silk fibroin (SF) was conducted. Facile solution casting of Silk Fibroin-Graphene Oxide hydrogels was used to prepare the composite. The high tensile strength of  $221 \pm 16$  MPa apart from high modulus of  $17.2 \pm 1.9$  GPa have been demonstrated by the composite film which contained 15% (by weight, wt %) of SF [56]. The strong hydrogen bonding between SF chains and GO sheets may also have contributed to the high mechanical properties of the composite film. Silk fibroin scaffolds was fabricated from a high concentration aqueous silk fibroin solutions [7]. Different initial concentrations of 8, 10, 12 and 16%, in wt. % were used to fabricate the SF scaffolds. The methodologies adopted to prepare the scaffolds were the combination of salt-leaching and freeze drying. The results have shown that the scaffolds comprised the antiparallel  $\beta$ -pleated sheet structure as well as macro/microsporous structure. Nevertheless, the mean porosity reduced from  $90.8 \pm 0.9$  to  $79.8 \pm 0.3\%$  and the mean connectivity reduced from  $97.4 \pm 0.5$  to  $92.3 \pm 1.3\%$  when the silk fibroin concentration was increased from 8 to 16%. However, the increment in the SF concentration has caused the dry state compressive modulus to be increased from  $0.81 \pm 0.29$  to  $15.14 \pm 1.70$  MPa while the wet state dynamic storage modulus to be increased approximately 20 to 30 fold at every single testing frequency. The study has also shown that even after the in vitro degradation for 30 days, the scaffolds still retained their structural integrity, morphology and stable mechanical properties. Thus, the scaffolds can be used in the meniscus and cartilage tissue-engineering scaffolding [7]. The properties of the silk fibroin extracted from the silk gland of 7-day-old fifth instar larvae of *B. mori* were explored [57]. The aqueous silk fibroin solution (ASF) was derived from the simple and eco-friendly method without using the harmful and toxic solvents or chemicals. From the study, when ASF concentration was increased from 2 wt% through 4 wt% to 6 wt%, the compressive strength increased from  $1.6 \pm 0.2$  Mpa through  $2.6 \pm 0.1$  Mpa to  $4.5 \pm 0.1$  Mpa. The compressive strength of these scaffolds was increased to  $2.1 \pm 0.2$  Mpa,  $3.6 \pm 0.4$  Mpa and  $6.9 \pm 0.4$  Mpa respectively after the treatment with ethanol. Nevertheless, the pore size reduces when the concentration of ASF was increased from 2 wt% through 4 wt% to 6 wt%. Hence, the lowest porosity at 6 wt% of ASF concentration may have contributed to the highest compressive strength. Silk hydrogel are now gaining attention as their biochemical composition and properties can be adjusted according to natural tissue. It can be used in bone and cartilage tissue engineering,

drug delivery and also for controlled release of DNA for gene therapy [58]. It was found that human MSC seeded on silk hydrogel increased from  $150 \pm 30$  cells/mm<sup>2</sup> to  $622 \pm 87$  cells/mm<sup>2</sup> [59]. Silk fibroin have been explored in bone tissue engineering [60]. Metabolic activity and proliferation of ATDC5 cells seeded on non- and cross-linked Eri fibroin silk sponges showed significant increment until 14 days of culture [61].

## **VI ADVANTAGES OF SILK**

Comprehensive research have been carried out over the past few years to formulate a biomaterial which is similar to the human body in terms of physical and mechanical properties. It is even more challenging to create a material which is both biocompatible and non-toxic to the human body. In the field of medicine, natural material is preferred compared to their synthetic counterparts. This is due to its outstanding mechanical properties. Silk's tensile strength is between 0.5 to 0.6GPa. Elongation of silk can reach up to 40% of its initial length [62]. All of these contribute to the toughness of silk [63]. Silk has remarkable strength to density ratio due to its strength and light-weight [64]. Another superior feature of silk is it can resist high level of stress and strain [62]. Mechanical properties is totally dependent on the type of silk [64]. Silk's excellent mechanical properties can be utilized in the biodegradability area of biomaterial.

Apart from excellent mechanical properties, silk has also good biocompatibility. Lewis rats exhibited mild immune response when 3D silk scaffold were implanted in them [65, 66]. Silk has also been used in the production of ligament in tissue engineering. The pig model showed no signs of adverse effect and the in-vivo culture did not malfunction [67]. Silk's biocompatibility is far more superior compared to collagen and PLA as it induced lower immune response [9]. In addition, silk has lower inflammatory response compared to polystyrene and poly (2-hydroxyethyl methacrylate).

Silk is both biodegradable and bio-resorbable [68]. Biodegradable is defined as the loss of mass of the material where the fragments can be moved from the implantation area but not completely from the body [69]. On the other hand, bio-resorbable is the ability to completely metabolise the by-products due to the degradation. Implantation of water-based scaffold in Lewis rats degraded within a few weeks and was completely metabolised within a year [66]. The by-products from the degradation imposed no harmful effects. Systems which uses silk can be prepared using water based solution which requires only mild conditions for manufacturing. These systems only require room temperature, neutral pH and without the need of high force shear. These conditions are very favourable for loading sensitive drugs onto silk implants [70]. These conditions also increases the ability of biosensor to bio-integrate in vivo [62]. By using water vapour annealing, mechanical stretching and ultrasonic treatment, resistance towards dissolution, thermal and enzymatic degradation can be induced in silk [63,71].

In addition to that, silk also exhibits excellent optical transparency in the visible range which is very beneficial to the biosensor field [55, 72]. Silk is economically viable as large amount of silk are produced for the textile industry.

## **VII LIMITATIONS OF SILK**

The disadvantage of the degumming process is that the process alters the mechanical properties of the silk which is not favourable [73, 74]. The long term effect of silk are still questionable. More comprehensive research needs to be carried out in order to observe the long term effect of silk in human body. A major issue in bio-implants is the generation of particulate debris which may induce inflammation [75]. Fractions from silk are able to prompt immune response [76]. Apart from that, enzymatic reaction on silk which degrades the silk could result in reduction of cyto-compatibility feature [77]. On the other hand, many researches which were carried out proved otherwise [78, 79]. Degradation of silk may result in amyloidogenesis which may subsequently result in the degradation of surrounding tissue [80].

## **VIII FUTURE WORK**

Silk has been extensively researched in numerous field specifically in medical field. For instance, silk is integrated in the design of a microchip which would aid in eliminating infections [81]. This microchip is created as such that it is dissolvable. It is made up of magnesium on a film of silk. The microchip is turned on with the assistance of wireless connection and the resistor in the microchip heats up. The heat would in turn eliminate the infections. Silk is also being developed as printable inkjet [89]. This would reform the future of wound healing. Wound dressing with drugs can be printed out according to the size and shape of the wound. The ink doubles as a contamination detector. Silk balls are being created as a treatment to arthritis. The silk balls are injected into arthritic joints as lubricants. Silk is being developed as vaccines which requires no refrigeration [82].

## **IX CONCLUSION**

Silk is a model biomaterial. It is one of the most diverse biomaterial available due to its remarkable properties for instance high mechanical strength, biocompatibility and biodegradable. The potential application of silk was studied primarily on tissue engineering and regenerative medicine. Numerous study fabricated 3

dimensional (3D) and porous silk scaffold adopting electrospinning method. The method produces nanofiber structure which mimic the extracellular matrix (ECM) of the cells. Nonetheless, more research is required for the silk to advance in the field of biomedical engineering.

## **X. CONFLICT OF INTERESTS**

The authors declare that there is no conflict of interest regarding the publication of this article. None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this paper,

## **REFERENCE**

- [1] Thilagavathi, G., &Viju, S. (2015). 11 - Silk as a suture material. In A. Basu (Ed.), *Advances in Silk Science and Technology* (pp. 219-232): Woodhead Publishing.
- [2] Das, S., Bora, U., Borthakur, B., &Kundu, S. (2014). Applications of silk biomaterials in tissue engineering and regenerative medicine. *Silk biomaterials for tissue engineering and regenerative medicine*. Woodhead Publishing Limited, 41-77.
- [3] Hakimi, O., Knight, D. P., Vollrath, F., &Vadgama, P. (2007). Spider and mulberry silkworm silks as compatible biomaterials. *Composites Part B: Engineering*, 38(3), 324-337. doi: 10.1016/j.compositesb.2006.06.012
- [4] Guinea, G. V., Elices, M., Pérez-Rigueiro, J., & Plaza, G. R. (2015). 10 - Structure and properties of spider and silkworm silk for tissue scaffolds\*. In A. Basu (Ed.), *Advances in Silk Science and Technology* (pp. 185-217): Woodhead Publishing.
- [5] Mortimer, B., & Holland, C. (2015). 12 - The use of spider silk as a biomaterial. In A. Basu (Ed.), *Advances in Silk Science and Technology* (pp. 233-260): Woodhead Publishing.
- [6] Zhao, J., Zhang, Z., Wang, S., Sun, X., Zhang, X., Chen, J., . . . Jiang, X. (2009). Apatite-coated silk fibroin scaffolds to healing mandibular border defects in canines. *Bone*, 45(3), 517-527. doi: 10.1016/j.bone.2009.05.026
- [7] Yan, L., Correia, C., Correia, J. S., Caridade, S., Fernandes, E., Mano, J., . . . Reis, R. (2012). Development of macro/micro porous silk fibroin scaffolds with nano-sized calcium phosphate particles for bone tissue engineering. *Journal of tissue engineering and regenerative medicine*, 6(Suppl. 1), 181.
- [8] Teuschl, A. H., Schuh, C. M., Halbweis, R., Marton, G., Pajer, K., Hopf, R., . . . Redl, H. (2015). Silk Fibroin for Peripheral Nerve Regeneration: A Novel Preparation Method Improved Mechanical Characteristics and Supports Regeneration in Rat Sciatic Nerves. *Tissue Engineering Part A*, 21, S36-S36.
- [9] Vepari, C., & Kaplan, D. L. (2007). Silk as a Biomaterial. *ProgPolymSci*, 32(8-9), 991-1007. doi: 10.1016/j.progpolymsci.2007.05.013
- [10] Shi, P., & Goh, J. C. H. (2012). Self-assembled silk fibroin particles: Tunable size and appearance. *Powder Technology*, 215-216, 85-90. doi: 10.1016/j.powtec.2011.09.012
- [11] Ratner, B. D. (2013). *The Biomaterials Literature*. 1491-1493. doi: 10.1016/b978-0-08-087780-8.00146-7
- [12] Abdel-Naby, W., & Lawrence, B. D. (2015). 9 - Processing of silk biomaterials. In A. Basu (Ed.), *Advances in Silk Science and Technology* (pp. 171-183): Woodhead Publishing.
- [13] Wang, Y., Kim, H. J., Vunjak-Novakovic, G., & Kaplan, D. L. (2006). Stem cell-based tissue engineering with silk biomaterials. *Biomaterials*, 27(36), 6064-6082. doi: 10.1016/j.biomaterials.2006.07.008
- [14] Wenk, E., Meinel, A. J., Wildy, S., Merkle, H. P., &Meinel, L. (2009). Microporous silk fibroin scaffolds embedding PLGA microparticles for controlled growth factor delivery in tissue engineering. *Biomaterials*, 30(13), 2571-2581. doi: 10.1016/j.biomaterials.2008.12.073
- [15] Li, C., Vepari, C., Jin, H. J., Kim, H. J., & Kaplan, D. L. (2006). Electrospun silk-BMP-2 scaffolds for bone tissue engineering. *Biomaterials*, 27(16), 3115-3124. doi: 10.1016/j.biomaterials.2006.01.022
- [16] Wang, X., Hu, X., Daley, A., Rabotyagova, O., Cebe, P., & Kaplan, D. L. (2007). Nanolayer biomaterial coatings of silk fibroin for controlled release. *J Control Release*, 121(3), 190-199. doi: 10.1016/j.jconrel.2007.06.006
- [17] Zhang, X., Reagan, M. R., & Kaplan, D. L. (2009). Electrospun silk biomaterial scaffolds for regenerative medicine. *Adv Drug Deliv Rev*, 61(12), 988-1006. doi: 10.1016/j.addr.2009.07.005
- [18] Feng, Z., Rong, L., Zuo, B. Q., & Qin, J. Z. (2010, 18-20 June 2010). Electrospun Silk Fibroin Nanofiber Tubes for Peripheral Nerve Regeneration. Paper presented at the Bioinformatics and Biomedical Engineering (iCBBE), 2010 4th International Conference on.
- [19] Liu, H., Li, X., Zhou, G., Fan, H., & Fan, Y. (2011). Electrospun sulfated silk fibroin nanofibrous scaffolds for vascular tissue engineering. *Biomaterials*, 32(15), 3784-3793.
- [20] Wang, Y., Kim, U. J., Blasioli, D. J., Kim, H. J., & Kaplan, D. L. (2005). In vitro cartilage tissue engineering with 3D porous aqueous-derived silk scaffolds and mesenchymal stem cells. *Biomaterials*, 26(34), 7082-7094. doi: 10.1016/j.biomaterials.2005.05.022

- [21] Jin, H. (2004). Human bone marrow stromal cell responses on electrospun silk fibroin mats. *Biomaterials*, 25(6), 1039-1047. doi: 10.1016/s0142-9612(03)00609-4
- [22] Min, B., Lee, G., Kim, S., Nam, Y., Lee, T., & Park, W. (2004). Electrospinning of silk fibroin nanofibers and its effect on the adhesion and spreading of normal human keratinocytes and fibroblasts in vitro. *Biomaterials*, 25(7-8), 1289-1297. <http://dx.doi.org/10.1016/j.biomaterials.2003.08.045>
- [23] Xie, H., Yang, W., Chen, J., Zhang, J., Lu, X., Zhao, X., . . . Wang, Z. (2015). A Silk Sericin/Silicone Nerve Guidance Conduit Promotes Regeneration of a Transected Sciatic Nerve. *Advanced healthcare materials*.
- [24] Permanent Waving Is Biochemical Engineering - Glucose Phosphate. (2016). [Barnardhealth.us](http://www.barnardhealth.us). Retrieved 2 April 2016, from <http://www.barnardhealth.us/glucose-phosphate/permanent-waving-is-biochemical-engineering.html>
- [25] Eisoldt, L., Smith, A., & Scheibel, T. (2011). Decoding the secrets of spider silk. *Materials Today*, 14(3), 80-86. [http://dx.doi.org/10.1016/s1369-7021\(11\)70057-8](http://dx.doi.org/10.1016/s1369-7021(11)70057-8)
- [26] Hardy, J., Römer, L., & Scheibel, T. (2008). Polymeric materials based on silk proteins. *Polymer*, 49(20), 4309-4327. <http://dx.doi.org/10.1016/j.polymer.2008.08.006>
- [27] Kawamoto, K., Kawamoto, T., Shiba, H., & Hosono, K. (2013). A histochemical study of the posterior silk glands of *Bombyx mori* during metamorphosis from larvae to pupae using frozen sections. *Biotechnic&Histochemistry*, 89(2), 145-152. <http://dx.doi.org/10.3109/10520295.2013.830777>
- [28] Heijenoort, J. (2001). Formation of the glycan chains in the synthesis of bacterial peptidoglycan. *Glycobiology*, 11(3), 25R36R. <http://dx.doi.org/10.1093/glycob/11.3.25r>
- [29] Millard, M., & Masri, M. (1974). Detection and analysis of protein functional group modification by x-ray photoelectron spectrometry. *Analytical Chemistry*, 46(12), 1820-1822. <http://dx.doi.org/10.1021/ac60348a039>
- [30] Eisenberg, D. (2003). The discovery of the  $\alpha$ -helix and  $\beta$ -sheet, the principal structural features of proteins. *Proceedings Of The National Academy Of Sciences*, 100(20), 11207-11210. <http://dx.doi.org/10.1073/pnas.2034522100>
- [31] Sakai, F., Yang, G., Weiss, M., Liu, Y., Chen, G., & Jiang, M. (2014). Protein crystalline frameworks with controllable interpenetration directed by dual supramolecular interactions. *Nature Communications*, 5, 4634. <http://dx.doi.org/10.1038/ncomms5634>
- [32] Doshi, U., & Muñoz, V. (2004). The Principles of  $\alpha$ -Helix Formation: Explaining Complex Kinetics with Nucleation-Elongation Theory. *The Journal of Physical Chemistry B*, 108(24), 8497-8506. <http://dx.doi.org/10.1021/jp049896a>
- [33] Fukushima, Y. (1996). Synthesis and Highly Stable  $\beta$ -Sheet Formation of Sequential Alternating Amphiphilic Polypeptides. *Polym J*, 28(2), 113-120. <http://dx.doi.org/10.1295/polymj.28.113>
- [34] Alberts, B. (2002). *Molecular biology of the cell*. New York: Garland Science.
- [35] Zhang, S., Pan, Q., Zhang, H., Zhang, Y., & Wang, H. (2003). Classification of protein quaternary structure with support vector machine. *Bioinformatics*, 19(18), 2390-2396. <http://dx.doi.org/10.1093/bioinformatics/btg331>
- [36] Inoue, S., Tanaka, K., Arisaka, F., Kimura, S., Ohtomo, K., & Mizuno, S. (2000). Silk Fibroin of *Bombyx mori* Is Secreted, Assembling a High Molecular Mass Elementary Unit Consisting of H-chain, L-chain, and P25, with a 6:6:1 Molar Ratio. *Journal Of Biological Chemistry*, 275(51), 40517-40528. <http://dx.doi.org/10.1074/jbc.m006897200>
- [37] Sirichaisit, J., Brookes, V., Young, R., & Vollrath, F. (2003). Analysis of Structure/Property Relationships in Silkworm (*Bombyx mori*) and Spider Dragline (*Nephila edulis*) Silks Using Raman Spectroscopy. *Biomacromolecules*, 4(2), 387-394. <http://dx.doi.org/10.1021/bm0256956>
- [38] Sehnal, F., & Žurovec, M. (2004). Construction of Silk Fiber Core in Lepidoptera. *Biomacromolecules*, 5(3), 666-674. <http://dx.doi.org/10.1021/bm0344046>
- [39] Boutry, C., Řezáč M., & Blackledge, T. (2011). Plasticity in Major Ampullate Silk Production in Relation to Spider Phylogeny and Ecology. *Plos ONE*, 6(7), e22467. <http://dx.doi.org/10.1371/journal.pone.0022467>
- [40] Madurga, R., Plaza, G., Blackledge, T., Guinea, G., Elices, M., & Párez-Rigueiro, J. (2016). Material properties of evolutionary diverse spider silks described by variation in a single structural parameter. *Sci. Rep.*, 6, 18991. <http://dx.doi.org/10.1038/srep18991>
- [41] Chen, G., Liu, X., Zhang, Y., Lin, S., Yang, Z., & Johansson, J. et al. (2012). Full-Length Minor Ampullate Spidroin Gene Sequence. *Plos ONE*, 7(12), e52293. <http://dx.doi.org/10.1371/journal.pone.0052293>

- [42] Guinea, G., Elices, M., Plaza, G., Perea, G., Daza, R., & Riekkel, C. et al. (2012). Minor Ampullate Silks from *Nephila* and *Argiope* Spiders: Tensile Properties and Microstructural Characterization. *Biomacromolecules*, 13(7), 2087-2098. <http://dx.doi.org/10.1021/bm3004644>
- [43] Adrianos, S., Teulã, F., Hinman, M., Jones, J., Weber, W., Yarger, J., & Lewis, R. (2013). *Nephilaclavipes* Flagelliform Silk-Like GGX Motifs Contribute to Extensibility and Spacer Motifs Contribute to Strength in Synthetic Spider Silk Fibers. *Biomacromolecules*, 14(6), 1751-1760. <http://dx.doi.org/10.1021/bm400125w>
- [44] Perry, D., Bittencourt, D., Siltberg-Liberles, J., Rech, E., & Lewis, R. (2010). Piriform Spider Silk Sequences Reveal Unique Repetitive Elements. *Biomacromolecules*, 11(11), 3000-3006. <http://dx.doi.org/10.1021/bm1007585>
- [45] Vollrath, F. (2006). Spider Silk: Thousands of Nano-Filaments and Dollops of Sticky Glue. *Current Biology*, 16(21), R925-R927. <http://dx.doi.org/10.1016/j.cub.2006.09.050>
- [46] Foo, C. W. P., Bini, E., Hensman, J., Knight, D., Lewis, R., & Kaplan, D. (2006). Role of pH and charge on silk protein assembly in insects and spiders. *Applied Physics A*, 82(2), 223-233.
- [47] Hueimmerich, D., Slotta, U., & Scheibel, T. (2006). Processing and modification of films made from recombinant spider silk proteins. *Applied Physics A*, 82(2), 219-222.
- [48] Kundu, B., Kurland, N., Bano, S., Patra, C., Engel, F., Yadavalli, V., & Kundu, S. (2014). Silk proteins for biomedical applications: Bioengineering perspectives. *Progress In Polymer Science*, 39(2), 251-267. <http://dx.doi.org/10.1016/j.progpolymsci.2013.09.002>
- [49] Das, S., Bora, U., Borthakur, B., & Kundu, S. (2014). Applications of silk biomaterials in tissue engineering and regenerative medicine. *Silk biomaterials for tissue engineering and regenerative medicine*. Woodhead Publishing Limited, 41-77.
- [50] Laurencin, C. T., & Freeman, J. W. (2005). Ligament tissue engineering: an evolutionary materials science approach. *Biomaterials*, 26(36), 7530-7536. doi: 10.1016/j.biomaterials.2005.05.073
- [51] Kim, H., Kim, U., Kim, H., Li, C., Wada, M., Leisk, G., & Kaplan, D. (2008). Bone tissue engineering with premineralized silk scaffolds. *Bone*, 42(6), 1226-1234. <http://dx.doi.org/10.1016/j.bone.2008.02.007>
- [52] Xie, H., Yang, W., Chen, J., Zhang, J., Lu, X., Zhao, X., . . . Wang, Z. (2015). A Silk Sericin/Silicone Nerve Guidance Conduit Promotes Regeneration of a Transected Sciatic Nerve. *Advanced healthcare materials*.
- [53] Das, S., Sharma, M., Saharia, D., Sarma, K. K., Sarma, M. G., Borthakur, B. B., & Bora, U. (2015). Data in support of in vivo studies of silk based gold nano-composite conduits for functional peripheral nerve regeneration. *Data in Brief*, 4, 315-321.
- [54] Teulé, F., Miao, Y.-G., Sohn, B.-H., Kim, Y.-S., Hull, J. J., Fraser, M. J., . . . Jarvis, D. L. (2012). Silkworms transformed with chimeric silkworm/spider silk genes spin composite silk fibers with improved mechanical properties. *Proceedings of the National Academy of Sciences*, 109(3), 923-928.
- [55] Liu, S., Dong, C., Lu, G., Lu, Q., Li, Z., Kaplan, D. L., & Zhu, H. (2013). Bilayered vascular grafts based on silk proteins. *ActaBiomaterialia*, 9(11), 8991-9003.
- [56] Huang, L., Li, C., Yuan, W., & Shi, G. (2013). Strong composite films with layered structures prepared by casting silk fibroin-graphene oxide hydrogels. *Nanoscale*, 5(9), 3780-3786.
- [57] Yang, M., Shuai, Y., He, W., Min, S., & Zhu, L. (2012). Preparation of porous scaffolds from silk fibroin extracted from the silk gland of *Bombyxmori* (*B. mori*). *International journal of molecular sciences*, 13(6), 7762-7775.
- [58] Kapoor, S. & Kundu, S. (2016). Silk protein-based hydrogels: Promising advanced materials for biomedical applications. *ActaBiomaterialia*, 31, 17-32. <http://dx.doi.org/10.1016/j.actbio.2015.11.034>
- [59] Floren, M., Bonani, W., Dharmarajan, A., Motta, A., Migliaresi, C., & Tan, W. (2016). Human mesenchymal stem cells cultured on silk hydrogels with variable stiffness and growth factor differentiate into mature smooth muscle cell phenotype. *ActaBiomaterialia*, 31, 156-166. <http://dx.doi.org/10.1016/j.actbio.2015.11.051>
- [60] Melke, J., Midha, S., Ghosh, S., Ito, K., & Hofmann, S. (2016). Silk fibroin as biomaterial for bone tissue engineering. *ActaBiomaterialia*, 31, 1-16. <http://dx.doi.org/10.1016/j.actbio.2015.09.005>
- [61] Silva, S., Oliveira, N., Oliveira, M., da Costa, D., Naskar, D., & Mano, J. et al. (2016). Fabrication and characterization of Eri silk fibers-based sponges for biomedical application. *ActaBiomaterialia*, 32, 178-189. <http://dx.doi.org/10.1016/j.actbio.2016.01.003>
- [62] Rajkhowa, R., Gupta, V., & Kothari, V. (2000). Tensile stress, strain and recovery behavior of Indian silk fibers and their structural dependence. *Journal of Applied Polymer Science*, 77(11), 2418-2429. [http://dx.doi.org/10.1002/1097-4628\(20000912\)77:11<2418::aid-app10>3.3.co;2-h](http://dx.doi.org/10.1002/1097-4628(20000912)77:11<2418::aid-app10>3.3.co;2-h)
- [63] Kundu, B., Rajkhowa, R., Kundu, S., & Wang, X. (2013). Silk fibroin biomaterials for tissue regenerations. *Advanced Drug Delivery Reviews*, 65(4), 457-470. <http://dx.doi.org/10.1016/j.addr.2012.09.043>

- [64] Ko, F., Kawabata, S., Inoue, M., Niwa, M., Fossey, S., & Song, J. (2001). Engineering Properties of Spider Silk. *MRS Proc.*, 702. <http://dx.doi.org/10.1557/proc-702-u1.4.1>
- [65] Cheung, H., Lau, K., Ho, M., & Mosallam, A. (2009). Study on the Mechanical Properties of Different Silkworm Silk Fibers. *Journal of Composite Materials*, 43(22), 2521-2531. <http://dx.doi.org/10.1177/0021998309345347>
- [66] Zafar, M., & Al-Samadani, K. (2014). Potential use of natural silk for bio-dental applications. *Journal Of Taibah University Medical Sciences*, 9(3), 171-177. <http://dx.doi.org/10.1016/j.jtumed.2014.01.003>
- [67] Wang, Y., Rudym, D., Walsh, A., Abrahamsen, L., Kim, H., & Kim, H. et al. (2008). In vivo degradation of three-dimensional silk fibroin scaffolds. *Biomaterials*, 29(24-25), 3415-3428. <http://dx.doi.org/10.1016/j.biomaterials.2008.05.002>
- [68] Fan, H., Liu, H., Toh, S., & Goh, J. (2009). Anterior cruciate ligament regeneration using mesenchymal stem cells and silk scaffold in large animal model. *Biomaterials*, 30(28), 4967-4977. <http://dx.doi.org/10.1016/j.biomaterials.2009.05.048>
- [69] Ho, M., Lau, K., Wang, H., & Bhattacharyya, D. (2011). Characteristics of a silk fibre reinforced biodegradable plastic. *Composites Part B: Engineering*, 42(2), 117-122. <http://dx.doi.org/10.1016/j.compositesb.2010.10.007>
- [70] Vert, M., Li, S., Spenlehauer, G., & Guerin, P. (1992). Bioresorbability and biocompatibility of aliphatic polyesters. *J Mater Sci: Mater Med*, 3(6), 432-446. <http://dx.doi.org/10.1007/bf00701240>
- [71] Karageorgiou, V., Meinel, L., Hofmann, S., Malhotra, A., Volloch, V., & Kaplan, D. (2004). Bone morphogenetic protein-2 decorated silk fibroin films induce osteogenic differentiation of human bone marrow stromal cells. *Journal Of Biomedical Materials Research*, 71A(3), 528-537. <http://dx.doi.org/10.1002/jbm.a.30186>
- [72] Wang, X., Yucel, T., Lu, Q., Hu, X., & Kaplan, D. (2010). Silk nanospheres and microspheres from silk/pva blend films for drug delivery. *Biomaterials*, 31(6), 1025-1035. <http://dx.doi.org/10.1016/j.biomaterials.2009.11.002>
- [73] Omenetto, F., & Kaplan, D. (2008). A new route for silk. *Nature Photonics*, 2(11), 641-643. <http://dx.doi.org/10.1038/nphoton.2008.207>
- [74] Perez-Rigueiro, J., Elices, M., Llorca, J., & Viney, C. (2002). Effect of degumming on the tensile properties of silkworm (*Bombyx mori*) silk fiber. *Journal of Applied Polymer Science*, 84(7), 1431-1437. <http://dx.doi.org/10.1002/app.10366>
- [75] Jiang, P., Liu, H., Wang, C., Wu, L., Huang, J., & Guo, C. (2006). Tensile behavior and morphology of differently degummed silkworm (*Bombyx mori*) cocoon silk fibres. *Materials Letters*, 60(7), 919-925. <http://dx.doi.org/10.1016/j.matlet.2005.10.056>
- [76] Purdue, P., Koulouvaris, P., Nestor, B., & Sculco, T. (2006). The Central Role of Wear Debris in Periprosthetic Osteolysis. *HSS Journal*, 2(2), 102-113. <http://dx.doi.org/10.1007/s11420-006-9003-6>
- [77] Altman, G., Diaz, F., Jakuba, C., Calabro, T., Horan, R., & Chen, J. et al. (2003). Silk-based biomaterials. *Biomaterials*, 24(3), 401-416. [http://dx.doi.org/10.1016/s0142-9612\(02\)00353-8](http://dx.doi.org/10.1016/s0142-9612(02)00353-8)
- [78] Minoura, N., Aiba, S., Higuchi, M., Gotoh, Y., Tsukada, M., & Imai, Y. (1995). Attachment and Growth of Fibroblast Cells on Silk Fibroin. *Biochemical And Biophysical Research Communications*, 208(2), 511-516. <http://dx.doi.org/10.1006/bbrc.1995.1368>
- [79] Baran, E., Tuzlakoğlu, K., Mano, J., & Reis, R. (2012). Enzymatic degradation behavior and cytocompatibility of silk fibroin-starch-chitosan conjugate membranes. *Materials Science And Engineering: C*, 32(6), 1314-1322. <http://dx.doi.org/10.1016/j.msec.2012.02.015>
- [80] Serban, M., Panilaitis, B., & Kaplan, D. (2011). Silk fibroin and polyethylene glycol-based biocompatible tissue adhesives. *Journal Of Biomedical Materials Research Part A*, 98A(4), 567-575. <http://dx.doi.org/10.1002/jbm.a.33149>
- [81] Kearns, V., MacIntosh, A., Crawford, A., & Hatton, P. (2008). Silk-based Biomaterials for Tissue Engineering. In *Tissue Engineering* (1st ed.). Sheffield: Centre for Biomaterials & Tissue Engineering, University of Sheffield.
- [82] Sheena McKenzie, f. (2015). Silk 'microchip' heals you from the inside - CNN.com. CNN. Retrieved 5 November 2015, from <http://edition.cnn.com/2015/06/19/tech/silk-microchip-science-healing-alternative-uses/>
- [83] STAT,. (2015). Spinning health solutions from silk - STAT. Retrieved 5 November 2015, from <http://www.statnews.com/2015/10/21/spinning-health-solutions-from->