

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 8, Issue 6, June 2019

Silk as a Biopolymer for Tissue Engineering Applications

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ABSTRACT: Regeneration of diseased or damaged tissues is one of the challenges in tissue engineering. Although there have been many methods developed, scaffold-based tissue engineering provides both biological and functional environment for regeneration. In last decade, Silk fibroin has gained immense importance for applications in tissue engineering scaffolding. It is a fibrous protein extracted from cocoons of *Bombyx mori* and spiders. It has been used as a biomaterial for tissue engineering owing to its unique mechanical properties, controllable biodegradation rate and biocompatibility. This review discusses about properties of silk fibroin and recent application with advancement of silk fibroin as a biomaterial in various tissue engineering approaches.

KEYWORDS: silk fibroin, biomaterial, tissue engineering, scaffold

I. INTRODUCTION

Majority of patients in the world are suffering from the consequences of trauma, injury, systemic disorders or other conditions like transplantation, surgical repair, and chemotherapy. In recent years, tissue engineering has emerged as most promising approach to replace diseased and damaged tissues through the development of artificial functional tissue constructs. There are full – furnished proofs of concept where tissue engineered transplants containing autologous cells have effectively been able to overcome the limitations associated with direct transplantation, that include graft rejection and disease transmission. Most importantly such an approach has reduced the requirement for organ donors. In order to treat the diseased tissue, the cells from normal tissues or organs (autologous or allogenic) are cultured in vitro on a scaffold to develop a functional tissue construct. The developed functional tissue construct can be transplanted inside the patient's body to overcome the diseased state. However, this field also faces few challenges which include engineering a suitable matrix with regenerative potential, delivery of signaling molecules etc. So, in this context, fabricating three-dimensional (3-D) scaffolds to form biocompatible and biodegradable materials with desired properties is essential to regulate vital cellular functions such as morphogenesis, differentiation, proliferation, adhesion and migration.

The principle of tissue engineering involves use of natural or synthetic polymer or a scaffold to make a three-dimensional living construct to replace the injured tissue. The scaffold made should be biodegradable and should be structurally, functionally and mechanically similar to or better than the tissue that is to be replaced. Among the various polymeric biomaterials, silk fibroin obtained from silk cocoon has been reported to be a potential candidate for tissue engineering. Silk is a natural polymer which is extracted from the cocoons of silkworm and is biocompatible, biodegradable with low immunogenic properties[1].

II. SIK AS A BIOMATERIAL

Silk is a natural polymer which is extracted from the cocoons of silkworm. It is a natural protein fiber which is available in abundant amount in the cocoon of Lepidoptera larvae such as silkworm (*Bombyx mori*) and a spider *Nephila clavipes*. The silkworms which are domesticated are Mulberry silkworms and these belong to the family Bombycidae. The silk obtained from mulberry silkworm is called mulberry silk. Commercially available form is obtained from only a single species called *Bombyx mori*.

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Scientific classification (according to Carolus Linnaeus, 1758):

Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Lepidoptera
Family: Bombycidae
Genus: Bombyx
Species: mori

Silk was discovered in china around 5000 years back and is produced through sericulture across Asia and Europe. The commercial interest of silk is not only in textiles but also in cosmetics and pharmaceuticals. The applicability of silk in biomedical sutures has thrown light on its utilization as bio-polymeric scaffold in tissue engineering. It also shows biocompatibility with the host tissue with its mechanical strength as a biomaterial. The viscoelastic property of biological tissues limits the application of synthetic polymers. This is because if the strength is increased it may compromise with the toughness of synthetic polymers. Among varieties of silk sources, silk from silkworm (*Bombyx mori*) and spider (*Nephila clavipes*) is used most extensively. The availability of *Bombyx mori* silk is abundant due to its farming, but not from *Nephila clavipes* because of its predatory nature. Spidroinin is a recombinant type of silk that is produced in host organisms. This paper deals with the silk protein from most common silkworm species, *Bombyx mori* and its application as a bio-scaffold for tissue regeneration.

Structure and Composition

Silkworm silk is produced from the cocoons of domestic moths, *Bombyx mori*. Silk fibers are 10-25 μm in diameter. Each fiber contains fibroin protein in the core coated with hydrophilic proteins called sericins (20–310 kDa) that keeps the core fibers together [2-5]. The core protein is made up of heavy chain (~390 kDa), light chain (~26 kDa) and a glycoprotein, P25 (25 kDa). The linkage between the Cys-C20 of heavy chain and Cys-172 of the light chain is of disulfide nature and joins the fibroin together and a glycoprotein of 25 kDa which is named P25 is attached to these proteins by non-covalent interactions [6, 7]. The importance of the light chain lies in the fact that it is essential for the secretion of protein from silk glands. P25 interacts with both heavy and light chains non-covalently. It assembles both heavy and light chains[8]. Heavy chain is the main fiber forming protein and its structure determines the properties of silk. It is commonly called the fibroin protein. The crystalline regions constitute 94% of the sequence and each repetitive region is on an average 413 residues long and consist of Gly-X repeats (X=Ala/ Ser/Thr/Val) forming hexapeptide sub-domains including: GAGAGS, GAGAGY, GAGAGA and GAGYGA. The most frequent (70%) occurring hexapeptide repeat sequence is GAGAGS. The crystalline repetitive region is responsible for the secondary structure (antiparallel β -pleated sheets) of the protein. Each crystal is on an average made up of 4 individual β -strands each being 11 amino acids in length. The β - strands are connected to each other by β - turns made up of GAAS amino acids[9, 10]. The high proportion (50%) of glycine, being a small amino acid, allows tight packing. This is why the fibers are strong and resistant to breaking. The tensile strength is due to the many inter seeded hydrogen bonds that play a crucial role when fibres are stretched, thereby resisting breakage by the applied force.

Mechanical Properties

The properties of silk are due to extensive hydrogen bonding, hydrophobicity and crystallinity. Silk biomaterials have an attractive balance of mechanical properties, such as modulus, breaking strength and elongation. These properties make silk a good candidate as a material of choice where toughness and ductility are desired properties in biomaterial. Silk fibers show higher toughness than Kevlar which is generally used as a bench mark in high performance fiber technology [11, 12]. Due to good strength and toughness of these silk fibers, these have been used in biomaterials to develop scaffolds for load bearing tissue engineering. The elastic modulus of *Bombyx mori* silk is 15-17 GPa and tensile strength is 610-690 MPa [13]. In tissue engineering, scaffolds provide an initial framework for the cells to attach and grow. The strength should be high enough to withstand initial loading conditions. Silk is much stronger than poly lactic acid (PLA) and cross-linked collagen, and will provide adequate support to growing cells.

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Biocompatibility

The *B. mori* silk have already been used as sutures and have been proved to be very efficient biocompatible biomaterials [14, 15]. However, since it is of non-mammalian origin sometimes it is shown to have concern about biocompatibility. The sericin protein which constitute about 25-30% of silk fibers has immunogenic properties [15, 16]. But the studies which involved isolated silk sericin protein have provided no clear evidence of sericin as the source of adverse reactions [17]. Numerous *in vitro* studies have demonstrated that once sericin is extracted, fibroin supports cell attachment and proliferation for a variety of cell types. *In vitro* evaluation of degummed fibroin demonstrates that the interactions of fibroin with the humoral components of the inflammatory system are comparable with those of polystyrene and poly (2-hydroxyethyl methacrylate), the two materials used extensively for biomedical applications [18]. Silk films implanted *in vivo* induced a lower inflammatory response than collagen films and PLA films [19].

Biodegradation

The degradation of biomaterials is important for tissue to regains its structure and function. An ideal tissue engineering scaffold should degrade at the rate which is similar to the rate of growth of the new tissue. This helps in integration of the engineered tissue into the surrounding host tissue and reduces the risk of stress shielding which is very important in case of engineering of tendons and ligaments. The silk fibroin fibers can retain more than 50% of their mechanical properties even after two months of implantation in the *in vivo* model, thus, they are considered to be non-degradable biomaterial [20]. The rate of silk fibroin degradation depends upon many factors like structure, morphology, mechanical and biological conditions at the implantation site. The studies have found that the implanted biomaterials of regenerated silk fibroin degradation is higher as compared to fibers and is dependent on the secondary structure of fibroin protein [21]. Degradation studies involving the systematic exposure of silkworm silk to enzymes have found that silk will degrade as a result of proteolysis with protease being reported to have most effect. Protease XIV from *Streptomyces griseus* is widely used enzyme followed by α -chymotrypsin from bovine pancreas for silk in *in vitro* models [22-24].

III. UTILITY OF SILK FIBROIN IN TISSUE ENGINEERING

An ideal scaffold for tissue engineering application must have the property of biocompatibility with host tissue, biodegradability and non-immunogenic nature. Therefore, much of the research is being done on natural polymer which fulfills these criteria. It proves to be an ideal polymer for tissue engineering application owing to its better bioactive properties as compared to synthetic polymers which is an important pre-requisite for a polymer for the development of construct. They can be easily modified in combination with other materials to achieve required mechanical and surface properties of scaffold. Therefore, silk is considered as a promising biomaterial and has been used for many tissue engineering applications as discussed below.

Skin Tissue Engineering

It has been found that silk fibroin coating allows and supports growth of dermal fibroblast cells and it do not show pro-inflammatory interleukins secretion [25]. Silk fibroin in the form of woven fibroin meshes also shows proliferation of oral keratinocytes which can be used as wound healing scaffold [26]. Silk materials in the form of films and sponges shows good wound healing response in *in vivo* conditions as compared to clinically used materials [27, 28]. The healing effect and toxicity of silk fibroin gel in skin wound of animal model and observed the accelerated epidermal (keratinocytes) cell proliferation, migration to wound and remodeling of wound site without any trace of cytotoxicity anatomical change showing its application in clinical trials of wound dressing application.

Silk fibroin has also been used as nanofibers as these are able to mimic the protein structure of ECM [29]. 3D silk fibroin nanofiber scaffolds as the ideal candidate for artificial skin reconstruction have been prepared [30]. Antimicrobial electrospun silk fibroin nanofibers have been prepared using silver nanoparticles and shown to have growth inhibition on *Staphylococcus aureus* and *Pseudomonas aeruginosa* [31]. 3D bi-layered artificial skin prepared from amniotic membrane with viscoelastic electrospun nanofibrous silk fibroin with autologous mesenchymal cells shown excellent cell adhesion and proliferation along with production of growth factors [32].

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Vol. 8, Issue 6, June 2019

Cartilage Tissue Engineering

Major research on the application of silk fibroin based 3-D scaffolds for cartilage tissue engineering were reported by Kaplan and his group, most often fabricated by salt-leaching [33-35]. *In vitro* human articular chondrocytes and mesenchymal stem cells (MSCs) cultured on these scaffolds have demonstrated chondrogenesis as elevated amount of collagen type II and GAG both in static [35] and dynamic [36] cultures. Hofmann and group studied the effect of serum in culture media in terms of support to chondrogenesis on silk and collagen scaffolds and reported that cartilage synthesis on silk scaffolds in serum-free conditions is highly significant. In the absence of serum, the GAG level was significantly higher on the fibroin scaffolds as compared to collagen controls but lower in the presence of serum, indicating the promotion of chondrogenesis due to surface properties of the silk scaffolds and its potential applications where the use of serum is avoided [37]. Silk fibroin sponges developed by thermally induced phase separation method showed support to chondrocyte proliferation for up to 28 days with inferior mechanical properties as of native tissue due to the inferior expression of synthesized extracellular matrix [38]. Alteration in cell culture protocol using the same scaffolds resulted in higher expression of type II collagen, GAG and extracellular matrix throughout the culture period of 28 days, signifying its application in hyaline cartilage tissue engineering [39, 40].

Bone Tissue Engineering

Above 2.2 million bone grafts are performed annually [41] and but have associated drawbacks and limitations due to the supply shortage, donor site morbidity and failure in specific regions [42, 43]. Silk fibroin biomaterials have also been used for bone tissue engineering due to its mechanical properties and it has fibrous nature similar to collagen type I with the amorphous links between the β -sheets in the structure which acts as sites for deposition of hydroxyapatite[44, 45]. Silk protein-based biomaterials have shown to improve the alkaline phosphatase (ALP) activity of osteoblast cells and osteogenic differentiation [46]. Furthermore, various signaling factors like bone morphogenetic protein/Transforming growth factor beta (BMP/TGF β), Wnt, Notch, fibroblast growth factor (FGF), platelet derived growth factor (PDGF) and insulin-like growth factor (IGF) regulate the osteogenic differentiation of stem cells on silk-based substrates[47].

Techniques have been developed to use 3-D porous silk fibroin scaffolds seeded with MSCs for the repair of bone defects with complex dimension. In a study, regenerated silk fibroin solution (RSF) was processed into films, electrospun to form scaffolds or a 3-D porous scaffold by salt-leaching. The addition of RGD functionality or growth factors was reported to enhance surface properties for cellular activities. Fibroin films coupled with RGD peptides incubated with osteoblast-like cells were found to produce mineralized matrix[48]. Silk fibroin films and scaffolds immobilized with bone morphogenic protein-2 (BMP-2) have been demonstrated to induce osteogenesis *in vitro* and bone formation when implanted with or without seeded pre-differentiated and undifferentiated MSCs in non-load bearing [49] and load-bearing [50] bone defect sites *in vivo*. Bone formation at the site of implantation with fibroin scaffolds seeded with undifferentiated cells *in vitro* induces early osteogenesis than scaffolds without pre-seeded cells. Silk fibroin scaffolds cultured with cells before implantation also has shown better mechanical properties [51]. Singh et al. fabricated silk fibroin/ Carboxy methyl cellulose scaffold using electrospinning and showed proliferation adhesion and differentiation of mesenchymal stem cells; and expressions of osteocalcin and type I collagen[52]. Silk fibroin/hydroxyapatite composites have also been tested for their ability restoring and repairing bone defects. Recently alginate, hydroxyapatite and silk fibroin composite for bone replacement have been prepared and shown to assist in bone repair [53].

Ligament and Tendon Tissue Engineering

The adaptability of silk biomaterial into controllable physical and mechanical properties makes it promising for ligament and tendon tissue engineering. Ligaments and tendons are both soft collagenous tissues composed mainly of fibroblasts. Ligaments connect bone to bone while a tendon connects the muscle to bone. Both are vital for movement and locomotion. Degummed *Bombyx mori* silk has been investigated for its potential applications in ligament tissue engineering. [54]. These matrices show suitable mechanical properties for ACL construction and modification to the yarn design and surface modification allows tailoring of the mechanical properties[55, 56]. Silk yarns have been found to allow growth and adherence of ligament fibroblast and MSCs [57], and these showed expression of ligament specific markers, although the cells did not infiltrate the center on individual cords[54]. When these cords were coated with RGD, the both cell types showed increased production of ECM and MSCs remain attached and aligned to the direction of mechanical stimulus. Cells were found to be rounded in absence of RGD and cells started to detach. The timing and

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the stimulus were very important and after nine days metabolic activity and collagen production was shown to increase[58]. When growth factors were applied to these MSCs on RGD coupled cords, it was found that the cell in-growth and collagen-I production increased but it altered the mechanical properties[59]. Coating of Bombyx mori fibers with gelatin restores mechanical properties which are lost by sericin layer removal so have been proposed for ligament tissue engineering [60].

Silk fibroin-based biomaterials have also been tested for tendon tissue engineering. Human tendon cells showed increased attachment and proliferation to silk-RGD films compared to unaltered and unmodified silk films and TCPS [61]. Level of mRNA for most abundant proteoglycan in tendon tissue which is decorin, and type-I collagen were found to be highest on silk-RGD and silk films as compared to control surface.

Vascular Tissue Engineering

Silk fibroin scaffolds are a possible alternative for vascular grafts due to combination of their unique mechanical properties to withstand vascular pulsating pressure and biocompatibility towards endothelial cells and smooth muscle cells. Soffer et al. fabricated electrospun nanofibrous tubular structures from silk fibroin with an objective to bioengineer small diameter vascular grafts[62]. Human endothelial cells and smooth muscle cells were able to successfully proliferate on substratum indicating their potential use as scaffolds for vascular grafts [63]. Scaffold porosity of 80% and above is reported ideal for endothelialization of vascular grafts [63]. Tubular silk scaffolds electrospun out of formic acid can resist up to 575 mmHg, which is much higher than physiological pressure of 120 mmHg [64].

Other Tissues

Studies have shown that collagen-fibroin blends and lactose silk fibroin conjugates support the attachment and proliferation of hepatocytes. It is also been reported that cells cultured on SF-containing films are able to eliminate ammonia and synthesize urea, suggesting potential applications for liver tissue engineering [65]. Silk fibroin has also been demonstrated to support the growth and proliferation of rat dorsal root ganglia (DRG) and Schwann cells on the substrate made up of purified silk fibroin fibers and has, therefore, been proposed as candidate for nerve tissue engineering[66].

IV. CONCLUSION

Silk fibroin is a natural biopolymer having chemical and physical properties suitable for biomedical application. It can be used with other biopolymers to create scaffolds for tissue engineering application. It is a protein polymer with tunable mechanical and degradation properties with biocompatible nature which makes it suitable for therapeutic applications. It has already been used for different tissue engineering applications like in bone and skin tissue engineering. With advances in processing techniques in scaffold production, new applications will be developed in the future and can open more possibilities in tissue engineering.

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Vol. 8, Issue 6, June 2019

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