Silk Protein based Novel Matrix for Tissue Engineering Applications

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Abstract

Tissue engineering deals with recreating, maintaining and enhancing the damaged or lost tissue/organ of living organisms. For tissue engineering applications, biomaterials are needed, that can be derived from either natural or synthetic source. Silk is one of the best natural biomaterials because of its properties like biocompatibility, high mechanical strength and slow degradation rate in vivo. Present study describes the synthesis of silk fibroin hydrogel using photopolymerization method. Aqueous silk fibroin solution was blended with synthetic polymer. The solution was then exposed by a monochromatic light source for the polymerization process. During the polymerization process, sample was at a specific distance from the light source under controlled temperature conditions i.e; at 37^oC. After the incubation period of around 3 hours, matrix with soft, slippery and elastic surface was obtained. The matrix obtained can further be used as a source for drug delivery carrier, for sustained delivery of drug, in the field of gene therapy for controlled release of DNA at the targeting site and also be used for bone and cartilage tissue engineering.

Keywords: Biocompatibility, Biomaterial, Degradation, Fibroin, Sericin, Silk Proteins, Tissue Engineering

1. Introduction

Tissue engineering merges the paradigms of engineering, cell biology and medicine to create matrices that can successfully mimic the histological, mechanical and morphological properties and substitute the natural function of tissues. Suitable scaffolds are needed to provide a 3D environment which can support proliferation and adhering of cells. For these tissue engineering applications, biomaterials are needed. Biomaterials can be derived from natural or synthetic material. Silk is one of the natural biomaterial. It is a naturally occurring protein polymer and is known for its properties like biocompatibility, strength and light weight and because of this, it is considered as one of the most suitable candidates for using as biomaterial in the field of tissue engineering¹. Silk includes a broad range of protein-based high molecular weight polymers often associated with insects, silkworm, and orb weaving spiders². Silk is a product of special insects that belong to the Lepidoptera order. The most extensively characterized silks are from the domesticated silkworm, *Bombyx mori*, and from spiders (*Nephila clavipes* and *Araneus diadematus*)³. Silk from *Bombyx mori* is a continuous strand of two filaments that are in a cemented form. This filament is a double strand of fibroin protein that is held together by another protein called sericin. Fibroin protein is responsible for unique physical and chemical properties of silk

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protein². Presence of sericin has been found to result in *in vivo* inflammation and therefore it needs to be removed from fibroin, a process called degumming, before further processing of silk protein⁴. Silk has a potential of getting fabricated into number of different forms such as fibers, films, gels, particles and sponges⁵. There are four structural components of silk protein; (i) Elastic β -spirals, (ii) Alanine rich crystalline β -sheets, (iii) amino acid repeats forming α -helices and (iv) spacer regions⁶.

Silkworm silk obtained from *B. mori* consists of light and heavy chains, with 25 and 325 kDa molecular weight respectively and are joined by single disulfide bond. Silk fibroin fibers that are obtained are generally 0.25-10 μ m in diameter with the presence of two proteins present at equal ratio(1:1)⁶. Along with these chains *B. mori* fibroin protein also contains glycoprotein named P25⁷.

Silk fibroin presents itself as a promising biomaterial candidate for many medical applications because of its unique structure, versatility in processing, nonimmunogenic response upon *in vivo* implantation, biocompatibility, availability of different biomaterial morphologies, ease of sterilization, thermal stability, surface chemistry for facile chemical modifications, and controllable degradation features. Tissue engineering and biomedical applications are explored because of its biocompatibility and biodegradability.

2. Structural Models of Silk Protein

Silkworm silk protein contains two different structural elements (Figure 1); Silk I: It is water soluble, and upon heat exposure or physical spinning gets easily converted to a silk II structure⁶. It contains mixed structures of alpha helices, type II β turn and random coil domains¹. The hydrophobic domains of β -sheet are generally asym-



Figure 1. Structure of silk protein⁹.

metrical and are occupied with hydrogen side chains from glycine on one side whereas on the other side, is occupied with the methyl side chains from the alanine. Arrangement of the β -sheet is such that the methyl groups and hydrogen groups that are present oppositely interact with each other to form the inter-sheet stacking in the crystals⁶. In the regeneration process of silk fibroins, silk I structure is easily converted to β -sheets using chemical methods (like treatment with methanol)⁸. Silk II: it is insoluble in solvents like water, mild acid and in alkaline conditions^{6,8}. It is composed of antiparallel β pleated sheets. The alignment of these β sheet content with the non-crystal domains of protein helps in determining the bulk mechanical properties and degradation kinetics of biomaterials that are generated from silk⁴.

3. Extraction of Silk Protein

Silk protein may be isolated from the cocoons or silk glands of silkworms. Various methods have been developed for the isolation of silk fibroin from the cocoons. Sericin is removed from the silk-worm cocoon by either sodium carbonate boiling or autoclaving process (with heat). Enzymes and other aqueous solutions are also useful in the extraction process¹⁰. The removal of this gummy like substance from the crude silk is entirely based on its solubility in hot water¹¹.

3.1 Isolation Process using Heat

Unlike fibroin, sericin is highly soluble in water and therefore, can be extracted in water by boiling silk fibres. Firstly, sodium carbonate in the diluted form is used for the removal of gum. Then the raw silk is extracted and the solution is further evaporated so as to get the powdered form. Alternatively, crude silk is boiled in water repeatedly by changing water several times until we get the precipitated form of the extract. Autoclaving for 3 h under 2.53 atmosphere pressure also helps in removal of this gummy like substance. For complete degumming, boiling solution of pH 11 with bentonite (5-6%) can be used. From the experiments, it was reported that the most constructive way for the removal is through autoclaving for one and half hour under pressure of 600-700 mm Hg (14 lb)².

3.2 Enzymatic Method

Enzymes are also helpful in the degumming process. For example; alkylase, alkaline protease can be used to degum fibroin (at 60°C for 90 min, at pH 10), while trypsin has been found to degum fibroin within 10 and 32 hours at 37 and 20°C, respectively. When 1 and 8 percent of trypsin solution is used for 4 h then, 26.4 and 28.7% of sericin is obtained, respectively¹⁰.

3.3 Extraction in Boiling Water

Small segments of *B.mori* silk cocoons of known weight are collected in a beaker and then washed with water by continuously stirring for 30 min at room temperature. Washed material is then boiled for 10, 20 and 30 min and fibroin is collected.

3.4 Alkaline Extraction

In this method, sodium carbonate solution is used for the extraction. Generally, 2.5 g of cocoons are cut into $1^{\times}1$ cm pieces and is then boiled in 1L of 0.02 M sodium carbonate solution for 1 h. Now the insoluble fibers are removed and then filtration process is carried out and finally evaporated to get the concentrated solution. Aqueous extraction in mild conditions (Anderlini method): Sodium azides (0.06%) are generally used. Solution containing 0.06% sodium azide, 50 ml water and 1 g of segmented cocoons are taken and then heated for 50 - 60°C with or without stirring¹². Removal of sericin is confirmed by scanning electron microscopy technique (SEM)¹³.

4. Properties of Silk Protein

4.1 Mechanical Strength

Silk protein fibre has excellent mechanical properties. It offers an attractive balance of modulus, breaking strength, and elongation, which contributes to its good toughness and ductility. It is found that silk fibers are tougher than Kevlar and their strength-to-density ratio is up to ten times higher than that of steel¹⁴.

4.2 Biocompatibility

In vitro studies revealed that silk in the film or fibre form do not show any macrophage activation. Silk is biocompatible because of its low inflammatory, immunogenic potential response and low cyto-toxicity³.

4.3 Degradation Behaviour

Degradation study of silk biomaterials is based on the mass loss, change in morphology and analysis of degraded products. Biodegradability is basically defined as action of biological elements that results in the degradation of implanted polymer giving rise to fragments of that implanted product which can then move away from the site through fluid transfer. But the degradation process is quite slow as silk biomaterials almost take days to several weeks to degrade in vivo. This property of silk can support the neo-forming tissues for long duration¹⁴. Silks are composed of β -sheet structures that are dominant in hydrophobic domain. It is usually considered as a degradable biomaterial over a longer period of time. This is because of the proteolytic degradation that is mediated by a foreign body response. Silk fibers generally loose majority of their tensile strength within 1 year in vivo8.

4.4 Thermal Stability

Silk proteins are highly stable at high temperature and thus sterilization of silk fibroin is feasible. Some applications require pre-sterilization of silk-fibroin solution like in the case of cell encapsulation in silk hydrogels, casting of silk sponges in plastic bioreactor systems. There are different techniques for sterilization like autoclaving, exposure of gamma radiation etc. Among this autoclaving is a simple and readily available sterilization technique¹⁵. Its high thermal stability and excellent mechanical properties makes silk compatible with common sterilization methods. This possibility of sterilization may help in reducing the manufacturing cost².

5. Medical Applications of Silk Protein

The fibroin based matrices can be utilized for a large number of applications (Figure 2)

- Hydrogel: Hydrogels have high water content that is beneficial for clinical use and are capable of absorbing large amount of water and thus maintains its structure^{7,10}. Hydrogels are able to swell in liquid solutions. As presence of water and a porous structure allows the passage of low molecular weight solutes and nutrients and also flow of waste out from the cell. The pore size of the network helps in determining the degree of transport through the hydrogel. Initially, gel should be in the form of fluid to fill-up irregular defects. It must resemble cartilaginous matrix and should facilitate cell infiltration and matrix production and have the ability to get degraded in the body by proteolytic enzymes¹⁰.
- In the form of scaffolds: Scaffold is a basic threedimension (3D) bioactive framework for cell activity and subsequent tissue regeneration. It provides an extracellular matrix for cultured cells to attach, grow and migrate. Therefore scaffold must be biocompatible. The scaffold should have porous architecture, which allows proliferation



Figure 2. Medical applications of silk protein¹⁶.

and migration of cells, diffusion of nutrients and oxygen, as well as formation of new capillaries. Scaffolds should also have the bioactivity to interact with host tissue facilitating and regulating the cell activities. Thus, depending on different tissue engineering applications scaffolds can be designed accordingly so that it matches the host tissue. Therapeutic cells pre-seeded in the scaffolds, facilitate cell regeneration and may also prevent any further damage to adjacent tissues following any injury¹⁷.

- For skin would healing: Fibroin films and fibroinalginate sponges have been reported to enhance skin wound healing *in vivo* compared to clinically used materials³.
- For vascular tissue engineering: Sulphonated and heparinised silk fibroin films are reported for use as artificial blood vessels by adjusting suitable mechanical properties. The studies showed that these films have good anticoagulant activity

and platelet response and support endothelial cell spreading and proliferation¹⁷.

- Sericin-fibroin blend film can be used to form artificial corneas. It can also prevent abrasive skin injuries and the development of rashes³.
- Silk is used as a coating drug carrying matrices with a potential of controlled release¹⁸.
- Silk fibroin has a wide range of application including burn-wound dressings, enzyme immobilization matrices, nets, vascular prostheses and structural implants⁸.

6. Methodology Used

Matrix was produced using silk protein mixed with natural compounds via photopolymerization mechanism keeping a specific distance between the monochromatic light source and the sample at 37°C temperature.

7. Result

Silk protein matrix was obtained by photopolymerization of silk fibroin solution. Matrix obtained was soft and elastic with slippery surface (Figure 3).

8. Conclusion

Silk Fibroin has been widely recognized as a suitable biomaterial because of its properties like high mechanical strength and excellent biocompatibility, slow degradation rate and high thermal stability. These characteristic features of silk protein enable it to be used for enormous biomedical purposes in the form of scaffold, hydrogel or porous microspheres for tissue engineering applications. In this work, we report a photopolymerization method to create silk hydrogel.



Figure 3. Silk protein matrix obtained by photopolymerization of fibroin solution.

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