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# STUDY ON SILK FIBROIN AS A BIOMATERIAL

Bipin Bihari Singh
Research Scholar, CMJ University
Supervisor Name: Dr. PP Singh
Reader of Zoology, A N College

**Abstract**

Degummed silk fibroin from *Bombyx mori* (silkworm) has potential carrier capabilities for drug delivery in humans; however, the processing methods have yet to be comparatively analyzed to determine the differential effects on the silk protein properties, including crystalline structure and activity.

***Keywords:*** silk fibroin, drug

## INTRODUCTION

Silk fibroin has been increasingly considered for biomedical applications due to its biocompatibility, low immunogenicity, slow degradation, versatility, and remarkable mechanical properties.

Silk fibroin can provide enhanced ability to remodel in response to the biological environment, leading to better integration and perhaps reduced material-associated thrombosis. This presents an attractive opportunity to exploit modified silk motifs as the basis for biomaterials with tailored properties. Specific amino acid motifs, historically correlated with functional properties, can be chemically and/or genetically modified while retaining critical secondary structural features, producing recombinant silk polymers that control

(1) Polymer size,
(2) Chemical reactivity, and
(3) Bulk material properties.

Unfortunately, pure silkworm silk fibroin poor hemocompatibility. Nevertheless, silk fibroin can be rendered anticoagulant via sulfonation, not surprising considering the chemical structure of the natural anticoagulant heparin. Recently, silk fibroin has been modified to improve hemocompatibility via addition of zwitterionic phosphobetaine, which demonstrated good nonthrombogenicity in the platelet adhesion assay. Additionally, ferulic acid silk fibroin, sulfonation, and heparin grafting showed that APTT (activated partial thromboplastin time), PT (prothrombin time), TT (thrombin time), and WBCT (whole-blood clotting time) were prolonged, indicating that modified silk may be an efficient anticoagulant.

Modification of silk fibroin with hirudin also improved hemocompatibility as indicated by limited platelet adhesion and aggregation by enhancing anticoagulation properties found in unmodified silk fibroin materials.

Defined silk amino acid motifs and periodicity also impart a level of control not possible with many traditional scaffold materials expanding their utility into a range of biomaterial and tissue engineering applications. Silk fibroin in various formats (films, fibers, nets, etc.) has been shown to support stem cell adhesion, proliferation, and differentiation in vitro and promote tissue repair and resist pathological adhesion in vivo.

Although the majority of previous work has been completed using silkworm silk, spider silks have superior mechanical properties, stemming from an expanded repertoire of specialized fibers and amino acid blocks allowing their sequences to be manipulated and genetically tailored with functional specificity. In 2014, Zhao et al. created a spider silk protein-based bilayer small-diameter vascular scaffold by compositing recombinant silk with chitosan, 42elatine, and PCL. Based on the material's recalcification coagulation time, the recombinant silk provided improved biocompatibility and hemocompatibility to the scaffold.

## LITERATURE REVIEW

Silk fibers produced by silkworms are widely used in our daily life. While they have occupied an important niche in the textile industry for thousands of years, their potential as biomaterials has been recognized and developed only over the past decade [1].

Being non-toxic, non-immunogenic, and biocompatible with a broad range of animal species has allowed for the adherent properties of silk fibroin and silk-like proteins to be exploited for biomedical purposes. To date, silk fibroins have mainly been applied to wound healing, successfully performing as man-made blood-vessels [2], surgical sutures [3], and repair materials [4].

New processing strategies for silk fibers and proteins have expanded the biomedical utility of these molecules. For example, the gel spun silk-based matrix derived from silk fibroin was successfully applied for bladder augmentation in a murine model [5].

More recently, scientists determined that the cocoons from *Bombyx mori* harbor antioxidant and hypolipidemic properties and that the crude silk extracts have bioactivity against hypercholesterolemia and atherosclerosis [6].

Recently, the regenerated silk fibroin has been proved as an attractive candidate of a carrier for drug or therapeutic proteins delivery and is the focus of much ongoing research. Attachment of bioactive molecules or therapeutic proteins to silk fibroin has many benefits to enhance the properties of bioactive molecules in solution for delivery both *in vitro* and *in vivo*, including the therapeutic efficacy in the body, thermal stability, storage stability, and lengthens the circulatory half-life and decreases immunogenicity and antigenicity [3].

For instance, bioconjugations of insulin, glucose oxidase, L-asparaginase (L-ASNase), lipase and phenylalanine ammonia-lyase with the regenerated silk fibroin greatly improved their biological stability, reduced the immunogenicity and toxicity of the drug [7-11].

Moreover, The SELP (silk-elastinlike protein polymer)-controlled gene delivery approach could potentially improve activity of adenoviral-mediated gene therapy of head and neck cancer and limit viral spread to normal organs at the same time [12].

It has been known that the properties of silk-matrix are controlled by a combination of the chemistry and the spinning process, which directly affect the activity and stability of the enzymes attached. Spinning conditions, such as temperature, drawing rate, time, and specific type of silkworm, can modulate biomaterial features. In addition, chemistry, such as ion concentration, type of ion, and solution pH, can also affect the mechanical properties of silk fibroins [1].

In previous studies, degummed fibroin has generally been treated with aqueous solutions of hexafluoro-isopropanol (HFIP) [13], methanol [8], CaCl2-ethanol [7,9], or Ca(NO3)2-methanol [14]. Lu et al. has reported glucose oxidase attached to the

regenerated silk fibroin film without treated with methanol remain more activity but lower stability than that treated with methanol [8].

After cross-linking L-ASNase with regenerated silk fibroin prepared with concentrated CaCl2 mixture solution with ethanol and water (1:2:8, mol), the immunogenicity and toxicity of the drug significantly reduced, and its circulatory half-life lengthened *in vitro*[9].

## MATERIAL AND METHODS

### *Materials*

L-asparaginase (L-ASNase) from *E. coli* (10,000 IU) was purchased from Changzhou Qianhong Bio-Pherma Co., Ltd. (Jiangsu Province, China). L-asparagines' (anhydrous) was purchased from Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, China). Trichloroactic acid (TCA) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Methanol, ethanol, calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$), calcium chloride ($CaCl_2$), and $HgI_2$, all analytical reagent grade, were purchased from Chengdu Kelong Chemical Reagent Factory (Sichuan Province, China).

### *Preparation of degummed silk fibroin*

Cocoons from *B. mori* were degummed by incubating in a mixture of sodium dodecyl sulfate (SDS; 0.25%,w/v) and sodium carbonate (0.25%,w/v) at 98°C for 30 min. The samples were then cooled to room temperature, rinsed three times with deionized water, and dried at 65°C overnight. The ratio of cocoons and solution was 1:100 (w/v). The degummed silk fibroins were isolated, along with another silk protein, sericin.

### *Calcium-alcohol solvents treatment of silk fibers*

The isolated fibroin fibers were separately dissolved in concentrated $CaCl_2$ solution mixed with ethanol or methanol and water (1:2:8 mol), and separately dissolved in concentrated $Ca(NO_3)_2{\cdot}4H_2O$ solution mixed with ethanol or methanol (1:2 mol) at 65°C in a water bath for 1 h. The ratio of the silk fibers and solution was 1:20 (m/v). The aqueous solution of silk fibroin was obtained by dialyzing against flowing water. After that, the resulting dialyzed solutions were lyophilized. The dry silk powder (fibroins treated with $CaCl_2$-ethanol solution) or pieces (from the other three solutions) were stored at 4°C until use.

### *SEM*

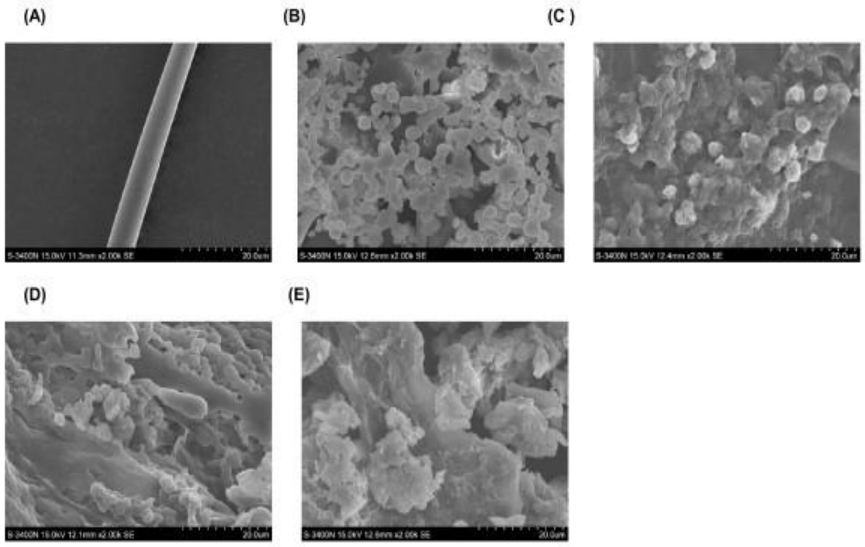
The silk fibroins were vacuum-coated with a 20 nm layer of gold. The surface morphology of each silk fibroin was observed with a scanning electron microscope (S-3400N SEM; Hitachi, Japan) and photographed at a voltage of 15 kV and room temperature.

## RESULTS AND DISCUSSION

Morphology of silk fibroins

The silk fibroins treated with $Ca(NO_3)_2{\cdot}4H_2O$-methanol, $Ca(NO_3)_2{\cdot}4H_2O$-ethanol, $CaCl_2$-methanol-$H_2O$, and $CaCl_2$-ethanol solution were separately dissolved. After lyophilized, the surface morphology of degummed silk fibroins and regenerated silk fibroins was observed with SEM (Figure (Figure1).1). The size and shape of the degummed silk fibroins were normal, with diameters of 6–8 μm (Figure (Figure1A).1A). In contrast, the regenerated silk fibroins were spherical or irregular shapes. This shape may have resulted from the merger of smaller micelles that occurred in the aqueous solutions of $Ca(NO_3)_2{\cdot}4H_2O$-methanol (Figure

(Figure1B),1B), $Ca(NO_3)_2 \cdot 4H_2O$-ethanol (Figure (Figure1C),1C), and $CaCl_2$-methanol-$H_2O$ (Figure (Figure1D),1D), and $CaCl_2$-ethanol (Figure (Figure1E).1E).




SEM photographs of B. mori silk fibroin prepared with various solutions. (A) Degummed silk fibroin. (B) Silk fibroin prepared from Ca(NO3)2·4H2O-methanol solution. (C) Silk fibroin prepared from Ca(NO3)2·4H2O-ethanol solution. (D) Silk fibroin prepared from CaCl2-methanol-H2O solution. (E) Silk fibroin prepared from CaCl2-ethanol-H2O solution.

**Molecular weight ranges of silk fibroins**

The silkworm's cocoon is composed of two kinds of silk protein, the silk sericin, which makes up the membrane, and the silk fibroin, which makes up the inner portion. The silk sericin is a glue-like mixture of glycoproteins with varying molecular mass, and is removed by the degumming and rinsing steps. The silk fibroin protein of B. mori is rich in alanine, glycine and serine residues [17], and is ~400 kDa, with 300 kDa making up a heavy chain (H-chain), 26 kDa making up a light chain (L-chain), L-chain and H-chain linked by disulfide bond(s) and about 30 kDa making up a P25 glycoprotein that associates with the H-L complex primarily by hydrophobic interactions [18].

**CONCLUSION**

The silk fibroins produced with Ca(NO3)2-methanol, Ca(NO3)2-ethanol, CaCl2-methanol, and CaCl2-ethanol solutions were dissolved, and the molecular weights were measured by SDS-PAGE. As shown in Figure , the regenerated silk fibroins treated with Ca(NO3)2-methanol had a molecular weight from about 95 KDa to over 170 kDa, but Ca(NO3)2-ethanol from about 100 KDa to over 170 kDa. The CaCl2-methanol solution fibroins ranged from about 140 to over 170 kDa, while the CaCl2-ethanol fibroins ranged from about 100 to nearly 300 kDa. Two low molecular weight bands, ~17 and ~26 kDa, were obviously present in these regenerated silk fibroins, but the silk fibroins produced with CaCl2-ethanol showed relatively faint low molecular weight bands at these positions. In addition, the degummed silk fibroins are poorly soluble, except in the chemistry solution and organic solvents, we could not observe obvious bands in the gel.

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