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SILK FOR BIOMEDICAL APPLICATIONS

Thirupathamma D[1]* Savithri G [1], and Kavya Sudha K [2]

[1]Department of Sericulture, S.P. Mahila Visvavidyalayam (S.P. Women's University), Tirupati -517502, Andhra Pradesh, India
[2] Narayana Pharmacy College, Nellore, Andhra Pradesh, India.

ABSTRACT

Silkworm produces a fine, glistening, fabulous proteinaceous silk thread the fabric made of which is regarded as the queen of textiles and has made great contribution to the human civilization. The silkworm, Bombyx mori produces massive amount of silk proteins during the final stage of larval development. Two kinds of silk proteins have been distinguished as major components of silk cocoons, the first being fibroin, a fibrous protein composed of heavy (H) chain, Light (L) chain and glycoprotein linked by disulfide bonds and the second being sericin a natural macromolecular protein, serving as an adhesive to unite fibroin for making silk cocoons of silkworm, B. mori. Recently, silkworm is being used as biofactory for the production of useful protein using the silk gland, which has promoted the technological development in sericulture. Silks have subsequently been investigated for use in Biomedical application and tissue engineering. In view of the significance of silk as biomaterial the present paper reviewed few important studies on biomedical applications.

Keywords: Bombyx mori, silk, Biomaterial, Biomedical.

Corresponding author

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INTRODUCTION

The *B. mori* is a lepidopteran molecular model and an important economic insect that is emerging as an ideal molecular genetic resource for solving a broad range of biological problems. The silk worm *Bombyx mori* has been domesticated for centuries and the silk so produced been used in textile manufacture and as suture material. Basically the silk is composed of two important proteins namely fibroin and sericin. The central fibroin core is coated with a covering of sericin. Silk protein is a kind of protein like collagen, elastin, keratin, fibroin and sporgin, and is an essential constituent of cocoon filament. The development of biomaterials is not a new area. It encompasses elements of medicine, biology, physical, chemistry, tissue engineering and materials science. Nevertheless, the demand for biocompatible, biodegradable and bioresorbable materials has increased dramatically since the last decade. An ideal biomaterial is one that is non-immunogenic, biocompatible and biodegradable, which can be functionalized with bioactive proteins and chemicals. In particular,biodegradability is one of the essential properties of the biomaterials. Natural biodegradable polymers like collagen, gelatin, chitosan and silk fibroin have promising advantages over synthetic polymers due to their favorable properties, including good biocompatibility, biodegradability and bioresorbability. Their physical and chemical properties can be easily modified to achieve desirable mechanical and degradation characteristics. Among these natural polymers, silk fibroin provides an important set of material options for biomaterials and scaffolds in biomedical applications because of its high tensile strength, controllable biodegradability, haemostatic properties, non-cytotoxicity, low antigenicity and oninflammatory characteristics. Hence, the paper reviewed significant contributions made on silk for biomedical applications.

SILK FOR BIOMEDICAL APPLICATIONS

Silks are naturally occurring polymers that have been used clinically as sutures for centuries. When naturally extruded from insects or worms, silk is composed of a filament core protein, termed fibroin, and a glue-like coating consisting of sericin proteins. In recent years, silk fibroin has been increasingly studied for new biomedical applications due to the biocompatibility, slow degradability and remarkable mechanical properties of the material. In addition, the ability to control molecular structure and morphology through versatile processability and surface modification options have expanded the utility for this protein in a range of biomaterial and tissue-engineering applications. Silk fibroin in various formats (films, fibers, nets, meshes, membranes, yarns, and sponges) has been shown to support stem cell adhesion, proliferation, and differentiation **in vitro** and promote tissue repair **in vivo**. In particular, stem cell-based tissue engineering using 3D silk fibroin scaffolds has expanded the use of silk-based biomaterials as promising scaffolds for engineering a range of skeletal tissues like bone, ligament, and cartilage, as well as connective tissues like skin. To date fibroin from *Bombyx mori* silkworm has been the dominant source for silk-based biomaterials studied. However, silk fibroins from spiders and those formed via genetic engineering or the modification of native silk fibroin sequence chemistries are beginning to provide new options to further expand the utility of silk fibroin-based materials for medical applications.

- Chen *et al* (2002) studied the effect of silkworm powder on type-2 diabetes mellitus for further development of anti-diabetic agent. The results showed that silkworm lowers blood-glucose of diabetes mellitus in animals effectively by the inhibition of □- Maltese glycosidase activity in the small intestine in order to delay the absorption of glucose by blood or tissues.

- Sericin is a highly hydrophilic protein family acting as the glue in *Bombyx mori* silk. In order to apply sericin as a wound dressing, a novel sericin film named gel film was prepared by a simple process without using any chemical modifications. Sericin solution was gelled with ethanol into a sheet shape and then dried. Infrared analysis revealed that the sericin gel film contained water-stable beta-sheet networks formed in the gelatin step. This structural feature rendered the gel film morphologically stable against swelling and gave it good handling properties in the wet state. The sericin gel film rapidly absorbed water, equilibrating at a water content of about 80%, and exhibited elastic deformation up to a strain of about 25% in the wet state. A culture of mouse fibroblasts on the gel film indicated that it had low cell adhesion properties and no cytotoxicity. These characteristics of sericin gel film suggest its potential as a wound dressing. Teramoto *et al* (2008)

- Silkworm silk fibers have been the primary silk- like material used in biomedical applications particularly as sutures. During decades of use, silk fibres have proven to be effective in many clinical applications. Tasubouchi (1999a) developed a silk fibroin- based wound dressing that could accelerate healing and could be peeled off without damaging the newly formed skin. The non-crystalline fibroin film of the wound dressing had a water content of 3-16% and a thickness of 10- 100 µm. Subsequently, the wound dressing was made with a mixture of both fibroin and sericin (Tsubouchi, 1999b). A membrane composed of sericin and fibroin is an effective substrate for the proliferation of adherent animal cells and can be used as a substitute for collagen. Minoura *et al.,* (1995) and Tsukada *et al.,* (1999) investigated the attachment and growth of animal cells on films made of sericin and fibroin. Cell attachment and growth were dependent on maintaining a minimum of around 90% sericin in the composite membrane.

- Already human growth hormone was successfully expressed through the silkworm larvae by the technology developed (Sumathy *et al* 1996). The purified human growth hormone thus isolated showed biological (radio immunological) activity indistinguishable from the authentic growth hormone. This high value, low volume product is of great biomedical importance in the treatment of dwarfism as well as in the healing of complex wounds.

- Recently, the regenerated silk fibroin has been proved as an attractive carrier for drug or therapeutic proteins delivery. 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. Preparation of *B. mori* degummed silk fibroin by $CaCl_2$-ethanol preserved the best original protein structure and produced a better affinity to the enzyme drug L-ASNase. The $CaCl_2$-ethanol solution may represent the most appropriate

method by which to prepare silk fibroins for use as biomaterials, especially as carriers for drug delivery. 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. 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 (Hao Zhang et al, 2012).

- The fibroin protein is one kind of biological materials used for artificial skin and other medical application. Silk fibroin membrane supports the application as photo sensor for hydrogen peroxide analysis. Silk protein sericin, suppress DMBA-TPA induced mouse skin tumor genesis by reducing oxidative stress, inflammatory responses and endogenous tumor promoter TNF-alpha (Zhaorigetu *et al.,* 2003).

- Silk fibroin films can be prepared by casting , spin coating , Langmuir-Blodgett (LB) and layer by layer deposition. Recently, patterned silk films have been developed as a cell supporting template for improved cell proliferation. High oxygen and water vapour permeability of silk films is important for their wound healing applications (Xun-Gai Wang et al, 2010).

- Mild aqueous processing of silk is advantageous for loading sensitive drugs without affecting their functions. For example, Lu *et al* , 2009 studied glucose oxidase, lipase, and horseradish peroxidise entrapped in silk films and found that for over 10 months, enzymes retained their significant activity, even when stored at 370C, and in the case of glucose oxidase, they did not lose any activity

- Silk has been used for the control release of specific drugs for targeted clinical needs. Uebersax *et al. 2006* and used silk matrices as adenosine-releasing bioincubators that may be useful in the management of epilepsy. Recently, Pritchard *et al 2010* demonstrated that slow (up to 2 weeks) and linear release of adenosine was possible by controlling the thickness and cystrallinity of silk coatings. Bayraktar *et al. 2005* used aqueous silk solutions to coat theophylline tablets. It was shown that slow release by multilayered silk coatings could enhance the efficacy of delivering emodin, an anti breast cancer and anti tumour drug [Cheema et al,2007,Gobin et al, 2006].

- Silk particles have also been examined for potential drug delivery applications. *Lorenz Meinel's* group reported encapsulation and release of horseradish peroxidase (HRP) using self assembled silk microspheres [Wang et al,2007]. They also reported encapsulation of salicylic acid, propranolol hydrochloride, and recombinant human insulin growth factor I (IGF-I) in silk microspheres.

- Kato et al. (1998) provided the first evidence of antioxidant action of the silk protein by showing that sericin suppressed in vitro lipid peroxidation. Furthermore, sericin also found to inhibit tyrosinase activity. These results suggested that sericin is the valuable natural ingredient for food and cosmetics. The biopolymer sericin has a strong affinity to keratin.

- silk–Polyethyleneglycol(PEG) biomaterial that stiffened the cervical tissue in vitro. This study is a first step toward a long-term goal of developing an injectable biomaterial as an alternative to cervical cerclage for treatment of a short cervix or cervical insufficiency (Asha et al, 2012).

- Film made of sericin and fibroin has excellent oxygen permeability and is similar to human cornea in its functional properties. It hoped that the sericin- fibroin blended film could be used to form artificial corneas (Murase et. al, 1994).

- Aslani and Eral, (1994) investigated the uranium recovery from dilute aqueous solutions using silk fibroin.

- The silk fibres can be arranged in parallel and optionally, intertwined (twisted) to form a construct; sericin may be extracted at any point during formation of fabric, leaving a construct of silk fibroin fibres having excellent tensile strength and other mechanical properties. The silk fibre is extremely hygroscopic which can absorb moisture up to 30% of its weight and still appear dry to the touch. Similarly, it exhibits elongation up to 35% and even in tensile strengths it is comparable to steel per unit weight. Unlike most visco-elastic fibres that arrive at a limit of break point, silk increases in strength, stiffness and elongation as the loading rate increases made them applicable in automobile industry . The use of natural fibre, silk as automotive parts improves environmental sustainability to some extent with reduced pollution (Manohar reddy , 2009).

- A novel method to fabricate silk fibroin hydrogels using high pressure carbon dioxide (CO_2) as a volatile acid without the need for chemical cross-linking agents or surfactants. The simple and efficient recovery of CO_2 post processing results in a remarkably clean production method offering tremendous benefit toward materials processing for biomedical applications. Further, with this novel technique we reveal that silk protein gelation can be considerably expedited under high pressure CO_2 with the formation of extensive β-sheet structures and stable hydrogels at processing times less than 2 h. We report a significant influence of the high pressure CO_2 processing environment on silk hydrogel physical properties such as porosity, sample homogeneity, swelling behavior and compressive properties. Microstructural analysis revealed improved porosity and homogeneous composition among high pressure CO_2 specimens in comparison to the less porous and heterogeneous structures of the citric acid control gels. The swelling ratios of silk hydrogels prepared under high pressure CO_2 were significantly reduced compared to the citric acid control gels, which we attribute to enhanced physical cross-linking. Mechanical properties were found to increase significantly for the silk hydrogels prepared under high pressure CO_2, with a 2- and 3-fold increase in the compressive modulus of the 2 and 4 wt % silk hydrogels over the control gels, respectively. We adopted a semiempirical theoretical model to elucidate the mechanism of silk protein gelation demonstrated here. Mechanistically, the rate of silk protein gelation is believed to be a function of the kinetics of solution

acidification from absorbed CO2 and potentially accelerated by high pressure effects(Michael et al,2012).

CONCLUSION

Silk from the silkworm *Bombyx mori*, has been used as bio medical suture material for centuries. Recently regenerated silk solutions have been used to form a variety of biomaterials such as gels, sponges and films for medical applications. Silk fibroin, fibres exhibit comparable biocompatibility in vitro and in vivo with other commonly used biomaterials such as polylactic acid and collagen. Silk scaffolds have been successfully used in wound healing and in tissue engineering of bone, cartilage, tendon and ligament tissues. Still there is lot scope to explore the silk as a biomaterial.

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