International Journal of Applied Agricultural Research
ISSN 0973-2683 Volume 12, Number 2 (2017) pp. 171-175

http://www.ripublication.com

The Silky Path of Biotechnology - A Review

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Abstract

Man had exploited silkworm for more than 5,000 years for the production of the proteinanceous silk fiber. Nearly, 8 million people in India cultivating silkworms for the production of silk and for their livelihood. Silkworms are known for production of the silk fiber, the finest of all natural and synthetic fibers. But not many know the utility of the silkworm for producing drugs for controlling diseases and other uses. Silk protein has a unique structure and chemistry that makes it strong, resistant to moisture, stable at extreme temperatures, and biocompatible, all of which make it very useful for stabilizing antibiotics, vaccines and other drugs. Make use of silk into micro needles to deliver a vaccine is an enormous added advantage that can potentially provide a lot of useful solutions to stabilization, distribution and delivery. One of the big challenges is supply and use of vaccines in remote regions where refrigeration is either limited, unreliable or non-existent as vital vaccines are sensitive to hot conditions. Too often vaccines fail to provide the necessary immune protection because the refrigerated supply chain that is supposed to protect them from temperature extremes while they are being transported has broken down. Nowadays cocoons are using as universal storage and as refrigerator. Many vaccines are developed from *Bombyx mori* for treating dread full disease like Measles, influenza , Hepatitis -B, flu vaccine etc and vaccines are also developed against Rabbit Hemorrhagic Disease (RHD), Neospora caninum (coccidian parasite), Bird flu etc in cattle. Research is also going on to develop vaccines for more dangerous diseases like Malaria, HIV etc. The production of vaccines by using silkworms is quicker and cost of production less costly than conventional methods. With this background the paper tries to discuss the utility of silkworm and silk as biological tool.

Keywords: Silkworm, Biological tool, Vaccines, Drugs

INTRODUCTION

Silk cocoon, the basic raw material of silk industry is considered as one of the highest dome of architecture and bioengineering in nature. The silkworm *Bombyx mori* produces massive amount of silk proteins during the final stage of larval development. Silk fiber consists of two types of self-assembled proteins *viz.,* fibroin and sericin. The central fibroin core is coated with an adhesive 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 demand for biocompatible, biodegradable and bioresourbable materials has increased dramatically since the last decade. Silks are naturally occurring polymers that have been used clinically as sutures for centuries. 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 on inflammatory characteristics.

The review summarizes the application of silk proteins with remarkable mechanical properties as biomaterial and emphasized the advantages and applications silk proteins as biomaterial.

- *Jeney Zhang et al (2012),* demonstrated selfstanding silk protein biomaterial matrices capable of stabilizing labile vaccines and antibiotics, even at temperatures up to 60 °C over more than 6 months. Importantly, these findings suggested a transformative approach to the cold chain to revolutionize the way many labile therapeutic drugs are stored and utilized throughout the world. Silk is composed of a block copolymer structure with large hydrophobic domains interspersed with small hydrophilic regions that form organized crystalline domains (β-sheets) that stabilize via physical cross links. This assembly forms nanoscale pockets that can immobilize bioactive molecules and improve their stability by minimizing water content and reducing protein chain mobility, thus preventing a transition from the native to denatured state. Extensive physical crosslinking, the hydrophobic nature of the protein and high glass transition temperature (around 178 °C) render silk highly thermodynamically stable to changes in temperature and moisture, and mechanically robust due to the heavily networked β-sheet structures. Due to its unique structure, encapsulation of therapeutic compounds in silk matrices could stabilize labile antibiotics and vaccines, akin to enzyme stabilization in silk .

- 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 form 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 the sericin gel film suggest its potential as a wound dressing. *Teramoto et al (2008)*
- Recently, the regenerated silk fibroin has been proved as an attractive carrier for drug or therapeutic protein delivery. Attachment of bioactive molecules or therapeutic proteins to silk fibroin has many benefits to enhance the properties of bioactive molecules in solution for delivering 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 *Bombyx 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 the 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).*
- *Tsubouchi et al (1999)* developed a silk fibroin based wound dressing material which accelerates healing and can be peeled off without damaging the newly formed skin. The non-crystalline fibroin film of the wound dressing has a water content of 3-16 % and a thickness of 10-100 µm. Several researchers studied the properties and application of wound protective membrane made of silk fibroin and concluded that, the fibroin membrane has good wound healing properties. Wound dressing can also be made with a mixture of both fibroin and sericin . A composite 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. Cell attachment and growth require a minimum of 90 % sericin in the composite membrane . Silk protein, sericin is having an antioxidant action which was proven by Kato and coworkers through *in vitro* lipid peroxidation studies.

- Silkworms have been used for recombinant protein production because of its high capacity of producing proteins and cost-effectiveness for large-scale production (*Kato et al., 2010).* Veterinary vaccines produced by recombinant baculoviruses are now on the market and some are under the development for licensing. In addition, subunit vaccines produced in silkworms are immunogenic and efficacious in cattle when used as prophylactic ones *(Li et al., 2008; Li et al., 2011*). Especially, in these cases, a hemolymph of silkworm larvae containing expressed the recombinant protein was used as a subunit vaccine against infectious disease in cattle. In this study, several antigens of *N. caninum* were expressed as FLAG-tagged proteins in silkworm larvae using BmNPV bacmid system.
- Kuniaki Nerome (2015) examined the possibility of preparing the VLP vaccine in silkworm and reported that significant amounts of influenza VLPs could be successfully produced in silkworm pupae by using a chimeric (multiple hybrid) HA DNA. A recombinant baculovirus was produced containing the synthetic H5 HA gene and was used to inoculate silkworm pupae for the mass production of the HA protein. Using this method, large amounts of influenza virus-like particles (VLPs) can be produced. In fact, the HA titers in homogenates from the infected pupae reached a mean value of 0.8 million HA units (HAU), equivalent to approximately 2,000 μg of HA protein per pupa, which is more than 50-fold higher than that produced in embryonated chicken eggs. Analysis of the VLPs in the purified HA fractions showed that they were attractive structures ranging from 30 to 300 nm in diameter. The spikes on the surface of the VLPs were approximately 14 nm long, densely packed, and were very similar to those on actual influenza virus particles. These VLPs reflected the characteristic structure of the influenza virosome and elicited immune responses that were much higher than those elicited by the MDP-liposome prepared in our previous study. Therefore, the use of chemical immunomodulators such as MDP should be considered in the future for the development of VLP vaccines.
- Silk is one of the oldest fibers known to humankind, has travelled a long way from china through silk Road and is a promising material for wide range of applications in the field of biomedicine as a biomaterial.

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