SILK PROTEINS IN BIO MEDICAL APPLICATIONS- A REVIEW

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ABSTRACT

Silk is a natural polymer synthesized and secreted by specialized silk gland of the silk worm. Silk is evolved as an ideal biomaterial can provide functional insight into relationships between polymer science and molecular biology. Silk proteins can be recovered from the silk during the processing chain of textile manufacture.The silkworm is being used as bio-factory to produce functional protein, promoting as a valuable biomaterial resource for modern applications .The superior moisture absorbance of silk fibre constructs bio-active textiles, while insulator and tensile properties substitute automotive and building construction applications. The silk sericin and fibroin proteins are prospective wound healing agents and are anti-oxidant and bio-adhesive mediators of human body. Beside its safety numerous studies have demonstrated its outstanding characteristics as a material for biomedical applications in the last few years. Especially its mechanical toughness/resilience based on the molecular structure, its slow rate of degradation and high processability have led to the use of silk in regenerative approaches of different tissues. Sericin protein can be cross-linked, copolymerized, and blended with other macromolecular materials, especially artificial polymers, to produce materials with improved properties. The protein is also used as an improving reagent or a coating material for natural and artificial fibers, fabrics, and articles.

KEYWORDS: *Silk, Natural fibre, Fibroin, Sericin, bio medical applications*

INTRODUCTION

Silk is generally defined as protein polymers that are spun into fibers by lepidoptera larvae such as silkworms, spiders, scorpions, mites and flies. Silk are fibrous proteins synthesized in specialized epithelial cells that line glands in these organisms. N.hyde silk fibroin polymers consist of repetitive protein sequences and provide structural roles in cocoon formation, nest building, traps, web formation, safety lines and egg protection. the most extensively used silk for various applications are those from silkworm silk; bombyx mori and spider silk; nephila clavipes.(S.b.dandin et al)The domesticated silkworm (b. mori) silk fibroin fibers are about 10–25 μm in diameter. Each fiber consists of core protein covered by a coating protein (sericin) that glues core fibers together. The core protein consists of three chains: heavy chain, light chain and a glycoprotein, p25. The light chain (26 kda) and heavy chain (390 kda) which are present in a 1:1 ratio and linked by a single disulfide bond. The disulfide linkage between the cys-c20 (20th residue from the carboxyl terminus) of the heavy chain and cys-172 of the light chain holds the fibroin together and a 25 kda glycoprotein, named p25, is non-covalently linked to these proteins. Light chain is necessary for the secretion of protein from the silk glands. Heavy chain is fiber forming protein and its structure determines properties

of silk fiber (arai et al 2004). Heavy chain is commonly referred as fibroin protein. These proteins are coated with a family of hydrophilic proteins called sericins (20–310 kda). Silk proteins are particularly promising for these needs due to their unique combination of biocompatibility, biodegradability, self-assembly, mechanical stability, controllable structure and morphology**.** Bombyx mori belongs to bombycidae produces a delicate twin thread of silk fibroin, which is coated by a protective cover of sericin. Silk protein is a kind of protein like collagen, elastin, keratin, fibroin, sporgin etc.,it is an essential constituent of cocoon filament (komatsu, 1975, 80). The silk fiber protein is synthesized by silk gland cells and stored in the lumen of the silk glands. Subsequently, it is converted into silk fibres. When the silkworms secrete the liquid silk during the spinning, it passes through the anterior gland and expelled out through the spinneret opening (Shimizu, 2000). Quantity and nature of sericin are fundamental characteristics in conferring distinctive traits sericin extracted from the liquid silk and fresh cocoon shells of a silkworm mutant, which secretes only sericin. Sericins from both the liquid silk in the silk gland and the cocoon filament and confirmed the relationship between solubility in hot water and molecular conformation (Komatsu, 1982).

Most experimental evidences indicate that sericin exists mainly in the random coil or β- structure. It is believed that β structure is intrinsic to liquid silk on analysis of circular spectrum, which, indicated that sericin extracted from liquid silk for 45 minutes with water contains a small fraction (10%) of α-helix. Komatsu (1980) argued that during the dissolution of the liquid silk in water, part of the sericin become a white suspension due to the coexisting cocoon yarn wax but does not coagulate and the β structure is originally present in the liquid silk. β-structure sericin is more insoluble than random coil sericin. The transition of sericin from its random coil to β- structure takes place by repeated absorption and de-absorption.

UTILITY IN BIOMEDICAL APPLICATIONS

Bombyx. Mori silkworm silk fibers have been the primary silk-like material used in biomedical applications particularly as sutures. During decades of use, silk fibers have proven to be effective in many clinical applications. at the same time, some biological responses to the protein have raised questions about biocompatibility. One of the major difficulties in assessing the biological responses reported to these silk fibers is the absence of detailed characterization of the fibers used including, extent of extraction of the sericin, the chemical nature of wax-like coatings sometimes used, and many related processing factors. This variability in source material has resulted in confusion in the literature and in clinical settings concerning the benefits or potential concerns with this class of fibrous protein.

APPLICATION OF SERICIN IN BIO MEDICAL

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)

The sericin composites are useful as degradable biomaterials, biomedical equipment and polymers for forming bioengineering articles, functional membranes and ligaments. in recent years, silk has been widely applied in native and reconstituted forms as nano fibre, film, hydrogel, membrane, sponge and particles for targeted biotechnological applications (kim et al 2005). The commercial production of sericin hydrolyzates as components of tissue culture media will expand its uses for tissue repair. The sericin can polymerize to 3-d structures that provide scaffolds for complex tissue reconstructions

Sericin can also be used as 8 % sericin cream which promotes wound healing with a significant decrease in wound area as compared to cream base treated wounds. The wound healing time is lesser in sericin cream (11 days) compared with the cream base treated wounds (15 days). Histological examination reveal that wounds treated with cream base show incomplete epithelization, ulceration and increased number of inflammatory cells than that of wounds treated with sericin cream

Use of the coated sericin film is a membrane composed of sericin and fibroinis an effective substrate for the proliferation of adherent animal cells and can be used as a substitute for collagen 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. 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 article corneas wounds.

A novel mucoadhesive polymer has been prepared by template polymerization of acrylic acid in the presence of silk sericin (Ahn et al. 2001). Silk protein can be made into a biomaterial with anticoagulant properties, by a sulfonation treatment of sericin and fibroin 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.

Sericin consists of about 30 % serine which is the main amino acid of natural moisturizing factor in human skin . Silk protein as a component of spongy sheets can also accelerate wound healing in rats by facilitating collagen synthesis . Sericin has more hydrophilic properties due to presence of several hydroxyl groups and so it is a better candidate for wound healing. Materials modified with sericin and sericin composites are useful as degradable biomaterials, biomedical materials, functional membranes, fibers and fabrics. Sericin, a major component of silk fiber is selectively removed from fibroin during the silk manufacturing process to make silk lustrous and the removed sericin goes as a waste material. Sericin protein is useful as a biomaterial because of its unique properties viz. Resists oxidation, antibacterial, UV resistant, absorbs and release moisture easily, inhibitory activity of tyrosine kinase etc from (Dandin et al 2007)

Sericin has good hydrophilic properties, it is also biocompatible and biodegradable, it actives the collagen production in wounds, and induces epithelialization (Aramwit et al., 2010; Sangcakul Aramwit, 2007). It is also reported that sericin promotes both attachment and proliferation of fibroblasts and keratinocytes in the human skin (Aramwit et al. 2013). These features allow its potential use as a wound healing agent. Aramwit & Sangcakul (2007) have made various tests using topical applications of sericin. They reported that cream with sericin powder (8% w/w) improvesscarring and reduces wound size in rats, without causing any allergic reactions (Aramwit & Sangcakul, 2007).

APPLICATION OF FIBRION IN BIO MEDICAL

The recent findings on the biocompatibility and biodegradability of fibroin have increased interest for fibroin as a biomedical material. Two growing areas of interest for fibroin are (1) as a material in contact lenses, and (2) for use in wound dressings. both applications require biocompatibility but also oxygen permeability (sashina et al., 2009; min et al., 2012)

There is considerable interest in fibroin as a material in wound dressings for not only its biocompatibility but also because the material itself has been found to enhance wound healing. Human skin fibroblasts, an essential part of the wound healing process, are able to attach to and grow on fibroin fibers to a level that is comparable to or even greater than that of collagen (Minoura et al., 1995).(Yamada et al.2004) used a proteolytic enzyme to digest fibroin and examined fibroblast adhesion and proliferation of the resulting peptide fragments. They found that two N-terminal region peptides are mainly responsible for increased fibroin growth, and the two peptides have a synergistic effect when they are together, further increasing fibroblast growth more than the individual peptides. Fibroin has also been shown to aid in the adhesion and growth of human keratinocytes, a skin cell responsible for reepitheliazation of wounds (Min et al. 2004). The adherence and growth of both fibroblasts and keratinocytes on fibroin should aid in the healing and healing time for wounds when used as a wound dressing.(Roh et al.2006) examined the healing capabilities of silk fibroin, alginate, and silk fibroin/alginate (SF/AA) blend sponges. These sponges as well as a gauze control were subjected to circular wounds surgically induced in rats, and the extent of regenerated epithelium, collagen deposition, and cell proliferation were examined. It was shown that these sponges significantly decreased wound healing time (by approximately 50%), increased collagen deposition, and increased cell proliferation compared to the gauze control. The SF/AA sponge also displayed slightly better healing capabilities than individual silk fibroin or alginate sponges. (Min et al.2012) showed that a silk fibroin sponge dressing impregnated with nano-Ag displayed impressive wound healing capabilities and antibacterial activity,

resulting in a decreased healing time of wounds as well as allowing for proliferation of a normal epidermal layer over the wound.

For regenerated fibroin films, the processing method will have an effect on the resulting polymer matrix and the resulting properties of the film. Treatment of fibroin films with methanol induces a change in the polymer matrix from an amorphous to a more crystalline structure dominated by anti-parallel β-sheets (Motta et al., 2002). Also it has been shown that the gas permeability of a material (e.g. oxygen, nitrogen, carbon dioxide permeability) changes as the material undergoes a structural change (Yasuda and Hirotsu, 1977). It is therefore important to examine the oxygen permeability of the fibroin films prepared using different methods, because differences in the structure or morphology of the polymer matrix will affect the gas permeability.

There are a few studies on the oxygen permeability of regenerated fibroin films. (Minoura et al.1990), who examined the dissolved oxygen permeability of silk fibroin films of varying crystallinities using an oxygen electrode, found that as the crystallinity of the film increased, the oxygen permeability decreased. At 20°C, the oxygen permeability ranged from 4-8 Barrer depending on the crystallinity of the film. (Kweon et al.2001) evaluated the dissolved oxygen permeability of silk fibroin/chitosan blend films, and they found that the oxygen permeability increased as the chitosan content in the film increased up to a maximum at 50/50 fibroin/chitosan, and then slightly decreased with a further increase in the chitosan content. The permeabilities of the pure fibroin and pure chitosan films were found to be 0.249 and 0.395 Barrer respectively, while the 50/50 fibroin/chitosan film was 0.579 Barrer.

Natural biopolymers tend to show both biocompatibility and biodegradability which are advantageous in controlled release systems. However, the material properties of many biopolymers cannot be modified sufficiently for most controlled release applications. For a material to be successful in implantable controlled release applications, it must possess a number of important material properties. The material must not only be biocompatible, it should also be biodegradable with no harmful degradation products. The biodegradability should be controllable. In addition, the material should have a compatibility with the drugs or biomolecules being delivered, and display controllable release kinetics (Pritchard and Kaplan, 2011). Silk fibroin appears to display all these properties, which makes it an ideal biopolymer for controlled release applications. Silk fibroin fibers and films display excellent biocompatibility and have unique and impressive mechanical properties (Altman et al., 2003). The mechanical properties of regenerated silk fibroin films can be modified by changing the crystallinity (Motta et al., 2002). Silk fibroin is biodegradable in vivo, has harmless degradation products of amino acids and small peptides, and the rate of biodegradation can be controlled from days to years through modification of the β-sheet crystallinity, processing solvent, silk concentration, and porosity (Lucas et al., 1957; Horan et al., 2005; Wang et al., 2005; Pritchard and Kaplan, 2011). Fibroin films and matrices are able to encapsulate and stabilize proteins and enzymes through intermolecular forces between the silk and biomolecules, then release the proteins undenatured and the enzymes with full activity (Lu et al., 2010). Silk fibroin can be processed and used in drug release in many different formats, including unprocessed fibers, films, nanolayers, hydrogels, sponges, and microspheres (Pritchard and Kaplan, 2011). The mass transfer and release kinetics within regenerated fibroin materials can be modified by changing the β-sheet crystalline content (Karve et al., 2011). Drug diffusion within the fibroin can also be controlled by the polymer morphology and other properties such as molecular mass (Pritchard and Kaplan, 2011). The drug release kinetics can also be modified by changing the degradation behaviour via the above mentioned, or through the co-release of proteinase inhibitors such as ethylenediamine tetraacetic acid along with the target drug to disrupt proteolytic activity (Pritchard et al. 2011).

One of the oldest uses for silk fibers was in medical sutures. Choi et al. (2004) showed that it is possible to enhance fibroin sutures using controlled release to create an infection resistant suture. Degummed silk fibroin was hydrolyzed, then two antibiotics, doxycycline and ciprofloxacin, were applied to the fibroin. The treated fibroin showed zones of inhibition of bacterial activity several centimeters in diameter for at least 24 hours in blood-flow simulated conditions. These results indicate the possibility of short-term infection resistant sutures, or even long-term infection resistant sutures that release antibiotics as the sutures degrade in vivo. Regenerated fibroin films have been investigated for controlled release using both matrix diffusion controlled and membrane permeation controlled systems. In MDC systems, the drug is added to the fibroin solution before the film is cast, and this provides a uniform distribution of the drug within the film matrix. Liu et al. (2009) loaded the anticoagulant drug heparin into a

polyurethane/silk fibroin blend membrane and examined the effects of film thickness, drug loading, and composition on the release kinetics. A longer sustained release of heparin occurred when membrane thickness was greater, when drug loading was higher, and for films with higher fibroin contents. For MPC systems, there has been research using both conventional films and nano-film coatings to surround the drug reservoir. Chen et al. (1994) examined the

permeability of a conventional fibroin film between two liquid reservoirs to five model drugs. The fibroin membrane was found to be an amphoteric ion exchange membrane, and would exclude one positively charged drug, while negatively charged drugs would have their permeability decrease with increasing pH, and the permeability of neutral drugs was independent of pH. The tendency for silk fibroin to form strongly interacting β-sheets allows for it to be deposited in a novel layer-by-layer nano-coating. This coating method can be utilized for controlled release applications.

Wang et al. (2007a) coated several drugs and proteins with a silk nanolayer and examined the release kinetics in vitro. The coatings displayed excellent mechanical properties for the application, and the release kinetics could be modified by controlling the β-sheet crystallinity of the coating as well as the coating thickness.

Fibroin hydrogels have also been created for controlled release applications. Hydrogels are a network of linked polymer chains that swell considerably when water is introduced, and have the ability to retain water in the polymer matrix. Fang et al. (2006) impregnated a fibroin hydrogel with the morphine-like opioid buprenorphine and examined the release behaviour. They found that the hydrogels followed a zero-order release rate, and the release could be controlled by changing the fibroin concentration. It was also noted that polymer drug solution can be injected and form stable hydrogel matrices once inside the body.

Silk fibroin shows remarkable mechanical, degradation and biocompatability properties, favoring its use to generate highly loaded grafts, especially in the musculoskeletal field (A. H. Teuschl, Nürnberger, Redl, & Nau, 2013)(Nau & Teuschl, 2015). In this regard, the studies on a silk fibroin- based anterior cruciate ligament (ACL) graft (Hohlrieder et al., 2013; Andreas Herbert Teuschl, van Griensven, & Redl, 2014) and its recent evaluation in a large animal study will be presented. Prior to this study a protocol to thoroughly remove the contaminating sericin from the silk fibroin based textile engineered scaffold has been established. This method is based on simple boiling steps of the raw textile-engineered scaffold in an alkaline borate buffered solution (pH 9.0), which is dissolving the sericin under preservation of the underlying silk fibroin with its desirable mechanical characteristics (Andreas Herbert Teuschl et al., 2014). In their study, were able to show that in contrast to novel method, the classical method to remove sericin based on sodium carbonate solutions does not work with such hierarchically complex fibrous structures as wire-rope designed scaffold. Scaffolds degummed in this way show mechanical properties similar to those of the native ACL tissue in terms of ultimative tensile strength as well as stiffness. For the testing of cell compatibility under mechanical straining a bioreactor system has been developed (Hohlrieder et al., 2013). In this system up to ten samples can be individually cultured at the same time and cyclically tensioned with defined longitudinal force for specific time patterns. In pre-vivo tests adipose tissue-derived stem cells have been cultured on the silk scaffolds and mechanically strained up to 3 weeks. These experiments showed that the cells stay viable on the scaffolds, colonize the whole scaffold under mechanical load and secrete a layer of ligament proteins (mostly collagen type I) on the silk fibroin fibers. After thorough in vitro testing, the material was also successfully applied in vivo. Since the first in vivo experiments in a rabbit model showed promising results in terms of excellent tissue compatibility and functionality also large animal studies in sheep have been performed. In these studies, the ACL of sheep has been replaced by the silk-based graft and the sheep have been observed for up to 12 months. After 12 months, the knee joints in all animals were stable and functional with no signs of damage to the surrounding knee structures such as menisci or hyaline cartilage which would occur in ACL-deficient knees. The silk-based graft had been completely invaded by cells from the adjacent tissues, which had partly degraded silk fibers and replaced them by ligament-like tissue. The results of this study have been recently accepted in the renowned American Journal of Sports Medicine (tentative publication date in June 2016).

In another study (Andreas Herbert Teuschl et al. 2015) we have created a textile-engineered so-called nerve guidance conduit also based on silk fibers. These types of scaffolds are intended to be used as guiding structures for regenerating peripheral nerves. The main tasks of these conduits should be acting as a barrier to invading fibroblasts from the surrounding tissue which might block nerve regeneration due to the formation of scar tissue, and the maintenance of a space for the regrowing nerves. For the generation of this structure, a protocol to fuse braided silk fibers to continuous layers has been developed. This process is based on the partial disintegration of silk fibroin molecules via the action of a ternary solvent consisting of CaCl2, ethanol and water in a molar ratio of 1:2:8. Thorough in vitro testing revealed that the silk nerve guidance conduits created show excellent cell compatibility, tested via the use of primary Schwann cells, and mechanical properties for a possible in vivo use. Subsequent in vivo tests clearly demonstrated that the silk based nerve guidance conduit enables the reconnection of peripheral nerves in a so-called gap model.

CONCLUSIONS

Silk from the silkworm Bombyx mori, has been used as bio medical suture material for centuries. Silk scaffolds have been successfully used in wound healing and in tissue engineering of bone, cartilage, tendon and ligament tissues. In modern society, many diseases have been increasing in human because of pollution, accident, lifestyle... The mutilation in human body leads to expand the needs of replacing tissues/ organs. However, the available source of tissues/organs is limited. Creating artificial tissues/ organs for replacing damaged, dysfunctional tissues/organs becomes a big discipline on material science. Although the current results have not completely satisfy the clinical demand, the potential applications of naturally derived biomaterials are still highly considered, therefore, research on this field have now being taken place all over the world.Still there is lot of scope to explore the silk proteins in bio medical applications.

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