Keratin and Sericin: State of the Art and Future Outlook

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ABSTRACT

Keratin and sericin are two major proteic biopolymers derived from renewable natural resources. Keratin is a hard protein characterized by presence of sulphur, mainly as the amino acid cystine. Keratin can be obtained from waste keratious materials such as feather, horns, nails, claws, hair and remnant of combing process of coarse wool. Sericin is discharged in the effluents of degumming of raw silk causing environmental problems as well as loss of possible income if properly utilized.

This article throws the light on the chemistry of keratin and sericin and summarizes methods to obtain them from renewable natural resources. The composites based on these biopolymers and their utilization in various fields is also outlined.

The current status and future outlook for utilization of keratin and sericin as well as composites there from is highlighted.

Keywords: Keratin, Sericin, Composite, Biopolymer

KERATIN

Keratin refers to wide-ranging class of insoluble proteins that associate as Intermediate Filaments (IFs) providing external covering such as hair, wool, feathers, nails, horns and hooves of mammals, reptiles and birds [1]. Covalent bonds, disulphide crosslinks and non-covalent interactions, which can occur between distinct polypeptide chains (intermolecular) and between different points of the same polypeptide chain (intra-molecular), are responsible for a highly stable structure of keratin fibre.

Keratin fibres, i.e., wool and human hair, consist of two major morphological parts: The cuticle layer (outer layer) which is composed of covering cells that surround inner part of the fiber. Cortex, the second part, includes a spindle-shaped cortical cell that which consists of non-keratinous proteins and lipids [2-5]. Keratin proteins classified into two groups: The intermediate filament proteins (IFPs) and the matrix proteins. The IFPs, α-keratin, are low in sulphur content while the matrix proteins, γ-keratin, are globular and have high content in cysteine, glycine and tyrosine residues [6].

The IFPs packing combined with the matrix proteins forming the macro fibrils within the cortex [7].

Another group of keratinous proteins, the β-keratin, formed the majority of the cuticle and their role is to protect keratin fibers from physical and chemical harm [8].

Extraction and applications of keratin

Many methods were developed to extract keratins from animal horns, hooves, wool and human hair using oxidative and reductive interactions [9,10].

Within the last decade, a group of researchers at the National Research Centre in Cairo extensively studied the possibility of dissolution of keratins in alkali metal hydroxide (lithium, sodium, or potassium hydroxide)or alkaline earth metal hydroxide (strontium or barium hydroxide) in presence of swelling agent (urea)and reducing agent (thiourea), for extraction of keratin [11-13].

In our opinion, using of this method in the presence of sodium hydroxide has been the best technology and economically technique for obtaining keratin from feather, wool and human hair.

It was mentioned that, the biological properties of the extracted keratin increasing interest for medical applications, where the first inventions were keratin powders for cosmetics, composites, and coatings for drugs [14].
Improving physical, chemical and biological properties of keratins has been done by fabrication keratin-based products such as films, sponges, scaffolds and fibers with excellent biocompatibility [10].

**Keratin films**

Solvent casting was the most appropriate technique for preparation of keratin films. The formed keratin films using solvent casting technique are characterized by their homogeneous thickness distribution, maximum optical purity, and extremely low haze beside the easily use of this technique [15].

Yamauchi et al. investigated the physicochemical and biodegradability of solvent-casted keratin films. It has been observed that, pure keratin films were too brittle for application but the addition of glycerol resulted in a transparent, relatively strong, flexible, and biodegradable film [16].

Incorporation of bioactive molecules as alkaline phosphatase into the keratin films, prepared form hair, was used as biomaterials in medical applications [17]. Furthermore, keratin have been studied for its purpose in bio material field such as for wound healing [18-22] bone regeneration [23], hemostasis [24] and recently in peripheral nerve repair [25,26].

The improvement of the physical strength and flexibility of keratin films has been motivated. This enhancement has been achieved by addition of natural [27-32] or synthetic [33-34] polymers to keratin forming new keratin films [35, 36] or by using crosslinking agents [37].

Chitosan has been applied to improve the mechanical properties of keratin films forming chitosan-keratin films with antibacterial properties, so it can be useful for cell culture [27].

In a different study, keratin-silk fibroin (SF) blend films have been prepared [29-33].Where, the antithrombogenicity and biocompatibility of the keratin-SF films was enhanced as compared to pure keratin and SF films, separately [30].

Keratin/poly (ethylene oxide) (PEO) film has been demonstrated to develop keratin materials for using as scaffolds for cell growth, wound dressings and drug delivery membranes [33].

In another study, keratin/polyamide 6 (PA6) blend has been established for creating keratin-based materials that applied in biomedical devices to active water filtration and textile fibers [34].

Compression molding of S-sulphokeratin powder technique was an alternative method to overcome the limited flexibility associated with solution-cast methods [36]. Since controlling the molding temperature and water content of the film controls the mechanical properties of the keratin films.

Reichl et al. have been developed films based on human hair keratin as substrates for cell culture and tissue engineering [38].

Ethylene glycol diglycidyl ether (EGDE) and glycerol diglycidyl ether (GDE) have been applied as crosslinking agents to improve the mechanical properties of keratin films that prepared by casting the reduced keratin solution [37].

Glycerol has been applied as plasticizer for superfine wool powder (with an average particle size of 1.7 µm) and hot-pressed into a thermoplastic film. The obtained films exhibited an improvement in the thermo plasticity and mechanical properties as well as in softness [39-40].

Blending keratin, from human hair, with gelatin for preparation of keratin/gelatin blend film has been studied for enhancing thermal properties of the keratin films [41]. This study showed that, gelatin assist to enhance some properties of keratin with remaining its strength.

It has been showed that, the utilization of human hair keratin, in a nano particle form, with addition of a softening agent followed by curing resulting in a biomechanical film that characterized for ocular surface reconstruction [42].

Wool was pretreated by TCEP (Tris (2-carboxyethyl) phosphine), as a reducing agent, for grinding into wool keratin powder by mechanical lapping. Subsequently, the formed keratin powder was dissolved in 88% formic acid, then by casting method keratin films were obtained with excellent moisture content [43].

Antimicrobial functionalization of keratin-based materials was obtained by chlorination reaction that resulted in the in situ formation of keratin-derived halamine compounds. Where, this process was providing bactericidal activity and biomedically interesting to keratin-containing materials [44].

Binary polymer blend films of alginate and keratin (from chicken feathers) were prepared by simple solution casting techniques. This study showed the formation of blend film with high enough tensile strength and suitable for a range of biomaterials such as for a drug delivery vesicle, hydrogel [45].

Furthermore, feather keratin films were used to load and release drugs. The resultant feather keratin biopolymer films showed good mechanical properties and controllable drug-release behavior [46].

Flexible, transparent, and appropriate cashmere-derived keratin-based films were obtained by simply environmental conditions during solvent-casting [47].

**Functionalization of textile with keratin**

It has been reported that, wool fabrics were treated with keratin hydrolysate to resist shrinking and improve strength [48]. Keratin}
has been extracted from cheap coarse Egyptian wool fibres using ammonium thioglycolate and the used for treating wool fabric in presence of epichlorohydrin as crosslinking agent to enhance its dyeability with acid and reactive dyes [49].

Electrospinning of soluble keratin

The electrospinning of nano fibers has increased interests of researchers in recent years because of the number of their potential applications in different areas, ranging from technical textiles such as, filters, composite reinforcements, and protective fabrics to biomedical commodities and devices such as bandages, membranes, bioactive surfaces, and porous substrates for tissue engineering, for which biocompatible polymers play an essential role. It has been reported that, Poly(ethylene oxide) PEO powder has been added to the keratin aqueous solution that obtained from wool by means of a sulphitolysis extraction method followed by electro-spinning into keratin:PEO nanofiber [50].

Keratinous materials, coarse wool or feather, has been dissolved in mixture of alkali metal hydroxide or alkaline earth metal hydroxide in the presence of swelling and reducing agents. The obtained soluble keratin then mixed with polyvinyl alcohol (PVA) and electro-spun into fibers [12].

SERICIN

Silk sericin

Sericin is a macromolecular protein covering the outer surface of raw silk fibron. Silk is composed of two main proteins; namely fibroin (fibrous protein), and sericin (globular, gumming protein).

Sericin is a protein [51-53] that consists of 18 amino acids [54], most of which have polar side chains including hydroxyl, carboxyl and amino groups (Figure 1) [55].

Being natural protein, sericin exhibits reasonable hygroscopicity by virtue of its various hydrophilic functional groups; namely hydroxyl, amino, and carboxylic groups.

A high hydrophilicity of sericin arises from the high content of serine and aspartic acid which constitute, about 33.4 and 16.7% of sericin, respectively (Figure 2) [55-57].

Sericin molecular weight (MW)

Sericin was listed as a family of proteins with a wide range of the molecular weight (MW) which is affected by different factors such as temperature, pH, processing time as well the extraction method [58].

The MW of sericin may be as low as 6 kDa or as high as 467 kDa based on the processing conditions [53]. Where, the high MW-sericin was suitable for making biomaterials and membranes whereas the low MW sericin was suitable for cosmetics, skincare products and medications [55].

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) has been applied to determine molecular weight distribution of acid-degraded and alkali-degraded silk sericin or that prepared by the high temperature and high pressure degumming technique [59].

HPLC system was used with a gel permeation chromatography (GPC) column to determine the MW of sericin [60]. Table 1 shows the different MW of sericin that reported in literature [55,61,62].

Up to our knowledge, sericin is not properly utilized and the discharged degumming wastewater causes environmental contamination due to the high oxygen demand for its degradation by microbes [63].
Sericin extraction and recovery

Sericin is removed and extracted from silk fibroin wall by a process known as degumming. Several methods were reported for degumming processes including extraction with water, boiling-off in soap [64], soda-ash method [65], degumming with alkalis and ultrasound method [66], degumming in acidic solutions [67], enzymatic method [68], plasma method [69] and microwave irradiation (Figures 3 and 4) [70,71].

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<th>MW (kDa) reported in literature</th>
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<td>10-300</td>
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**Table 1: Different MW of Sericin that reported in literature**

**Figure 3: Scheme of degumming of silkworm cocoon**

**Figure 4: Sericin extraction and recovery**
In our view, the degumming by applying microwave irradiation process is, technologically and economically feasible, the easiest and applicable process.

Different techniques have been reported for recovery of sericin from silk wastewater. Ethanol was applied for precipitation sericin, but this technique has no economically and environmental friendly impact when applied on the industrial scale [53]. Additional techniques were applied for sericin recovery such as enzymatic hydrolysis [72], freeze- and tray-drying [72], and membrane filtration [63,73] which is more benefit as compared to the other techniques because it provides an opportunity to separate sericin from other impurities.

One of the recent achievements of our team is the recovery of sericin from the degumming effluent by adjusting the pH of the bath at the isoelectric point and hence precipitation of sericin as a Zwitter ion (Figure 5).

\[
\text{HOOC–sericin–NH}_3^+ + \text{H}_2\text{O} = \text{HOOC–sericin–NH}_2^+ + \text{OH}^- \\
\text{Sericinium ion} \quad \text{Zwitterion} \quad \text{Sericinate ion}
\]

**Figure 5: Effect of pH on the electric charge of sericin**

In our view, the degumming by applying microwave irradiation process is, technologically and economically feasible, the easiest and applicable process.

**Silk sericin applications**

Industrially, sericin is removed from the silk fiber causing severe environmental pollution in receiving water bodies due to their rich organic contents [73]. It has been reported that, the production of silk cocoons is 600,000 tons/year, from which 150,000 tons of sericin can be recovered [75].

Sericin is a valuable protein with an economical value, so it has a variety of end-uses in cosmetics, pharmaceutical and biomedical industries.

Sericin has several biological activities and has proven to be a biocompatible agent. Serine with aspartic acid is responsible for sericin hydrophilicity and its sensitivity to chemical modifications. Sericin has unique biochemical and biophysical properties; namely biocompatibility, biodegradability, antibiotic-antibacterial activity, antioxidant performance, anti-tyrosinase activity, anti-carcinogenic effects, UV protective properties, and coagulant [57,76-79]. Moreover, the high content of serine and glycine make sericin to be used as a moisturizer in the cosmetics industry [77] and also enhances the elasticity of the skin as well as has anti-wrinkle and anti-aging effects via its collagen promoting activity [80,81].

Due to its hydrophilicity that assists to maintain a moist environment and to absorb excess exudates from wounds, sericin has been used as a wound dressing agent [80,82]. Sericin in two-dimensional form, like films and membranes or in three-dimensional form, like hydrogel and porous scaffolds matrices have been studied [83]. Sericin can form a film, casting film or gel analogue.

Genipin, a natural compound extracted from gardenia fruit, has been successfully cross-linked to sericin forming a scaffold with good physical properties [91].

Because thermal cross-linking of sericin-containing materials seems to be an unsuitable advance [84], on the other hand, the use of long wavelength light might be suitable for sericin crosslinking owing to the reaction is rapid and a small amount of biomolecules absorb light out of the UV region [83].

It was reported that, extracted sericin from silk waste was incorporated with glucomannan, polysaccharide polymer, and glycerol orming a flexible film [92].

Acrylonitrile was copolymerized with sericin to prepare a protein containing synthetic polymer film that used for separating water from organics [79,93].

Additionally, Thai silk sericin/polyvinyl alcohol (PVA) blending films were prepared that characterized by their high wet ability. By studying the contact angles of wetting fluids on surfaces of prepared film, it was found that contact angles decreased with contact time. Consequently, the Thai silk sericin/polyvinyl alcohol (PVA) blending films can be used in the development of a drug delivery materials [94].

Silk sericin films were prepared by using different polymers which are polyvinyl alcohol (PVA), polyoxyethylene-polyoxypropylene block copolymer (Plu) and glycerine (G) using cast method. The humidity absorption test of the obtained films showed the highest moisture absorption rate for film that prepared by using PVA [95,96].

Recently, Silk sericin/chitosan composite film was prepared by mixing different ratios of silk sericin and chitosan resulting in homogeneous composite film that has wound dressing applications [97].

Up to date, recovering and using sericin from degumming waste, for preparing sericin-contained biomaterials still need further exhaustive investigation.
Functionalization of textile with sericin

In textile field, sericin has been used for the fictionalization of natural and synthetic fibers to enhance water absorbency and smoothness [98,99].

Sericin has been applied to modify polyester fibers by crosslinking with glyceryl polyglycidyl ether and diethylenetriamine [100]. Sericin has been found to improve moisture regain and water retention, and reduce electrical resistivity of cotton fibers by using dimethyloldihydroxyethylene urea (DMDHEU) as crosslinking agents [101].

Cotton fibers have been also modified with sericin by using N,N'-dimethyl-4,5-dihydroxyethylene urea (DMeDHEU) and glutaraldehyde as crosslinking agents in a pad-dry-cure process to improve its comfortability and application for the development of medical textiles, for instance, gauze and pad dressing as their high moisture absorbency and smooth surfaces [98].

Polyester fabric has been functionalized and treated with sericin to improve its multifunctional properties and enhance its dyeability, wicking and moisture regain [102]. They also showed improved antistatic, ultraviolet protection and radical scavenging activity. These properties make sericin-treated fabrics suitable for use as medical textiles in wound dressings and for healing abrasive skin injuries in patients suffering from atopic dermatitis, pressure ulcers and rashes [103].

**FUTURE OUTLOOK**

Thio groups can be anchored along protein macromolecules such as keratin or sericin. The said protein-thiocyanate composite can be utilized for quantitative determination iron III cations in domestic or industrial water. Double decomposition reaction can be conducted between any protein and a thiocyanate salt in acid medium as follow (Figure 7):

However, this reaction mechanism should be controlled to avoid thiohydation reaction between protein and thiocyanate salt. This reaction is commonly used for detection of C-terminal protein sequencing. This can be achieved by reaction of amino acids with acetyl chloride as activating reagent and trimethylsilyl isothiocyanate (TMS-ITC) as derivatizing reagent (Figure 8).
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REFERENCES


