Short Communication

Influence of ethanol post-treatments on the properties of silk protein materials

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Abstract
The use of silk proteins sericin (SS) and fibroin (SF) has been increased for biomedical applications. In order to improve their behavior inside of biological environment, silk biomaterials must be treated after their manufacturing by means of diverse methods. These include ethanol post-treatments to increase their crystallinity, mechanical properties and water stability. In this study, the effect of ethanol post-treatments on the properties of silk protein materials was evaluated. Defective cocoons and silk fibrous waste (SW) were used to obtain silk sericin sponges (S-SS) and silk fibroin films (F-SF), respectively. Two ethanol treatments were evaluated in SS and SF: immersion (I) and solvent vapor annealing (SVA). Morphological modifications induced by ethanol post-treatments were studied by scanning electron microscopy (SEM). Conformational structure of the samples was analyzed by attenuated total reflectance–Fourier-transform infrared spectroscopy (ATR–FTIR), and the thermal properties were evaluated by differential scanning calorimetry (DSC) measurements. SEM images revealed that ethanol process induces changes in treated F-SF and S-SS, the material surfaces are more roughness, and these effects were more pronounced in samples treated by I than that subjected to SVA. As a result of the ethanol treatments, the ATR–FTIR and DSC results showed an increment in relative content of β-sheet structures in both silk protein materials. The results suggest that ethanol post-treatments induce conformational transitions and morphological modifications in S-SS and F-SF that should be considered to select the post-treatment conditions according to the biomedical application requirements.

Keywords Silk sericin · Silk fibroin · Ethanol treatment · Morphology · Structural properties

1 Introduction

Silk is a fibrous protein secreted by some insect and spider species to build natural structures for anchoring, feeding or protection purposes. For many centuries, the silk from the cocoon of the domesticated silkworm *Bombyx mori* has been used in the production of high-valued yarns and textiles due to its texture, luster, tensile qualities, comfort and its ability to take up dyes [1, 2]. This natural fiber consists of two filaments of silk fibroin (SF), a highly structured fibrous protein, coated with sericin (SS), which is a globular glue protein that acts as a binder to maintain the structural integrity of the cocoon [3]. Moreover, it has small amounts of waxy substances, mineral salts and coloring matter [4].

SF is widely used in the textile industry; instead, SS is generally discarded as a waste during a textile process called degumming [5], in which fibroin is separated from sericin. Both proteins have proven to have important biological properties, including biocompatibility and biodegradability; it also has been demonstrated to

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promote cellular adhesion and proliferation, which makes them potentially useful in different applications [6–9]. SF represents the 70–75% of the cocoon, and it consists of a light polypeptide chain (~ 26 kDa) and a heavy chain (~ 390 kDa), linked together by a disulfide bond [10]. The heavy chains in fibroin are composed of 12 hydrophobic domains separated by 11 hydrophilic domains. The hydrophobic domains comprise a repetitive sequence of amino acids that mainly include glycine (43%), alanine (30%) and serine (12%), and to a lesser extent tyrosine, valine and threonine [11]. On the other hand, SS accounts for 25 to 30% of the silkworm cocoon and it is formed by 18 amino acids, mostly of which have polar side chains such as hydroxyl, carboxyl and amino groups, which can cross-link, co-polymerize and be combined with other polymers [12]. Likewise, this protein is composed of 45.8% of hydroxy amino acids, 42.3% of polar amino acids and 12.2% of nonpolar amino acids [7], the highest proportion being serine, aspartic acid, glycine and threonine [13]. SS is composed of 70% hydrophilic amino acids, which provide it the ability to be partially soluble and to absorb large amounts of water [14].

Biomaterials synthesized from silk proteins can be manufactured in different forms, such as gels, sponges [15], films and fibers [16, 17]. The structural conformation or the relative content of secondary structures of these silk protein-based materials can be controlled during processing or with the application of post-treatments. Hence, it is possible to engineer silk biomaterials with tunable degradation rates, mechanical and other specific properties [9, 18, 19]. Due to its versatile properties and biological activity, silk protein materials have been extensively used in the research and development of biomaterials for diverse applications: wound healing [20–23], bone tissue regeneration [24] and vascular grafting [25]. However, materials fabricated using SF or SS as the only constituent generally have relatively weak structural and mechanical properties, which limit their extensive study in the biomedical field. Therefore, treatments by immersion in organic solvents like ethanol or methanol are commonly used to induce conformational changes in SS and SF that lead to a major stability; other techniques like solvent vapor annealing have been less explored [26, 27]. The use of ethanol for post-treatments can be advantageous compared to methanol, because it does not leave traces in the material and can serve as a sterilization method. Although later treatments with ethanol vapor are not well studied, it offers advantages such as the enrichment of crystalline structures with a minor change in morphology.

In this study, different ethanol treatments were used to induce structural and morphological modifications in SF films (F-SF) and SS sponges (S-SS). SF and SS were obtained from silk production by-products: silk fibrous waste and defective cocoons, respectively. The effect of ethanol treatments on the morphological and structural properties of silk protein materials was evaluated. Thus, it could be possible to design processes that derive more stable and diverse silk protein materials for biomedical applications.

2 Materials and methods

Defective cocoons (DC) and fibrous silk waste (SW) were supplied by the *Corporación para el Desarrollo de la Sericultura del Cauca*—CORSEDA (Cauca, Colombia). DC include perforated, double, deformed and stained cocoons with fine tips; meanwhile, SW comprises rejected cocoons, non-reeling parts of the cocoon, and other wastes that are not used during the manufacturing of textile products. For the degumming and solubilization of the SF, analytical-grade sodium carbonate (Na_2CO_3) and lithium bromide (LiBr) were used, respectively (Sigma-Aldrich, St. Louis, MO).

2.1 Extraction of SS and preparation of SS sponges

The defective cocoons were cut into small pieces to remove the dry pupa and some impurities. Sericin was extracted using the degumming technique at high temperature and pressure with hot water. An autoclave (All American, Model 25x-1, USA) was used for the extraction at temperature of 120 °C for 30 min, in a bath ratio of 1:30 (g of cocoons/mL distilled water). The sericin solution obtained was subsequently filtered using a vacuum pump to discard remaining cocoon pieces and to eliminate particulate material and possible impurities present in the solution. Next, a spray drying was performed, maintaining inlet temperature of 160 °C, spray flow of 40 m^3/h and flow rate of 6.3 mL/min. SS powder obtained was dissolved in distilled water at concentration of 2% (w/v) using an autoclave at the same conditions mentioned above. The solution obtained was poured into plates with 2-mL wells, which were frozen at − 80 °C for 24 h [28–30]. Then, the frozen samples were lyophilized for 48 h in a Labconco freeze dryer (USA).

2.2 Extraction of SF and preparation of SF films

The silk fibrous wastes were extracted following the procedures previously published [19, 31]. Briefly, the fibrous wastes were degummed in an aqueous solution of 0.5% (w/v) Na_2CO_3 at boiling for 30 min. SF obtained was filtered, dried at 60 °C for 24 h and subsequently dissolved using 9.3 M LiBr solution at 60 °C, and the resulted SF suspensions were dialyzed and filtered. SF obtained aqueous

solution after dialysis process was used to prepare F-SF by solvent casting at 35 °C until constant weight.

2.3 Ethanol post-treatments

In order to induce conformational and morphological modifications in silk protein materials, one set of samples were immersed in ethanol (S-SS/I and F-SF/I) and other were placed in saturated ethanol vapor environment (S-SS/SVA and F-SF/SVA). Both treatments were performed for 1 h. After this time, the samples were deposited in a desiccator for 24 h. The conditions selected for the treatments were based on the previous research carried out by other authors [32–34].

2.4 Characterization of SS sponges and SF films

The treated (with immersion and ethanol vapor) and untreated materials were subjected to the following characterization tests:

2.4.1 Scanning electron microscopy

The morphology of the materials was examined by SEM in high vacuum with a secondary electron detector (JEOL JSM-6490LV, USA). Measurements were performed on the cross-section cut of SS sponges and on the surface of SF films. Each sample was covered with a thin gold coating using Desk IV equipment (DENTON VACUUM), until a thickness of approximately 10 nm was achieved. The acceleration voltage for capturing the images (×100 and ×50) was 5 kV.

2.4.2 Fourier-transform infrared spectroscopy

The chemical structure of the materials was analyzed using the FTIR technique with attenuated total reflectance (ATR) module in a Nicolet 6700 Series spectrometer, USA. A total of 64 scans were performed at a resolution of 4 cm^{-1} and wavelength of 4000–400 cm^{-1}. Deconvolution was performed in the amide I region (1600–1700 cm^{-1}) using OMNIC software with Gaussian curve adjustment for the determination of secondary structures [35–37].

2.4.3 Differential scanning calorimetry

DSC curves for the samples were drafted using a Q2000 TA Instruments unit, USA, to study the thermal behavior of the samples. There were heated in aluminum crucibles in nitrogen atmosphere at flow rate of 50 mL/min, at temperature range of 30–320 °C, with constant heating rate of 10 °C/min [19].

3 Results and discussion

3.1 Scanning electron microscopy

The SEM micrographs obtained for SS and SF samples are shown in Fig. 1. Both untreated F-SF surfaces and cell walls

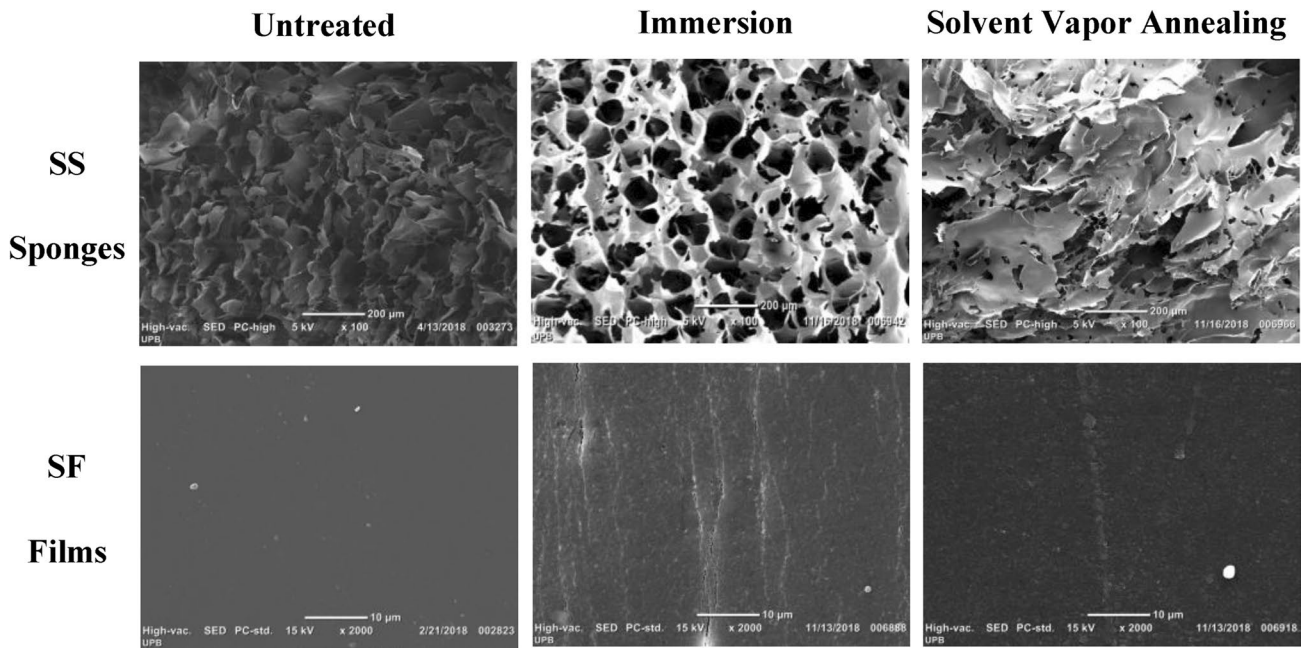

Fig. 1 SEM images of SS and SF materials

of S-SS exhibit smooth appearance. Untreated SS material also presents honeycomb structure with heterogenous size porosity [38]. Morphological changes seem to be more pronounce in samples treated by I than those treated by SVA; immersion in ethanol produces more rounded pore geometry in S-SS/I and generates rough and fractured surface in F-SF/I.

These results suggest that ethanol post-treatments produce contraction in the protein material, phenomenon explained by the hydrophobic dehydration that occurs due to molecular interactions between protein chains and the polar solvent [39].

3.2 Fourier-transform infrared–attenuated total reflection spectroscopy

Structural changes induced by ethanol treatments were studied by quantitative analysis of IR spectrum using deconvolution in the amide I region (1595–1705 cm^{-1}). This methodology is widely used to study conformational changes of protein materials due to its high sensitivity to small variations in molecular geometry and hydrogen bonding patterns [40–42]. According to previous studies, this area of the spectrum was adjusted to 19 and 12 Gaussian curves for SS and SF samples, respectively [35, 36, 43]. The relative content of different secondary structures in the protein materials is shown in Fig. 2. Compared with both treated and untreated SF films, SS sponges seem to have more crystalline state with higher content of β-turns, β-sheets and α-helix and lower presence of amorphous structures like random coil and side chains. This behavior is due to the transformation of random coil into β-sheets with the treatments applied, since β-sheet formation is induced by rearrangement of hydrogen bonds during the interaction between water molecules and the organic solvent [44].

Treated samples showed an increase in content of β-sheet crystalline structures, being more evident for the samples treated by immersion: S-SS/I (46.3%) and F-SF/I (32.28%). This can be attributed to the fact that the material was in direct contact with the solvent, so the SF molecules and the solvent molecules would have more interaction compared with the vapor treatment. Relative decrease in the proportions of random coil structures was found in treated samples. These results agree with conformational transition mechanisms reported by other authors, which involve the formation or highly ordered and stable β-sheet, while random coil and less ordered β-turn features tend to decrease [45]. Moreover, Chancow et al. [46] report an increase in the relative content of β-sheet by 10–12% in SF films treated with ethanol, coinciding with the values obtained in this study. Other authors also reported the increase in these crystalline structures by means of the displacement of the bands in the spectra obtained by FTIR [47, 48].

Results suggest that β-sheets formation is favored by the dehydrated state of the protein materials during the treatment. There, ethanol takes water molecules from the protein, producing strong interaction between them than favors its rearrangement in regular array. This results in conformational transitions from amorphous to more crystalline structures [12, 49, 50].

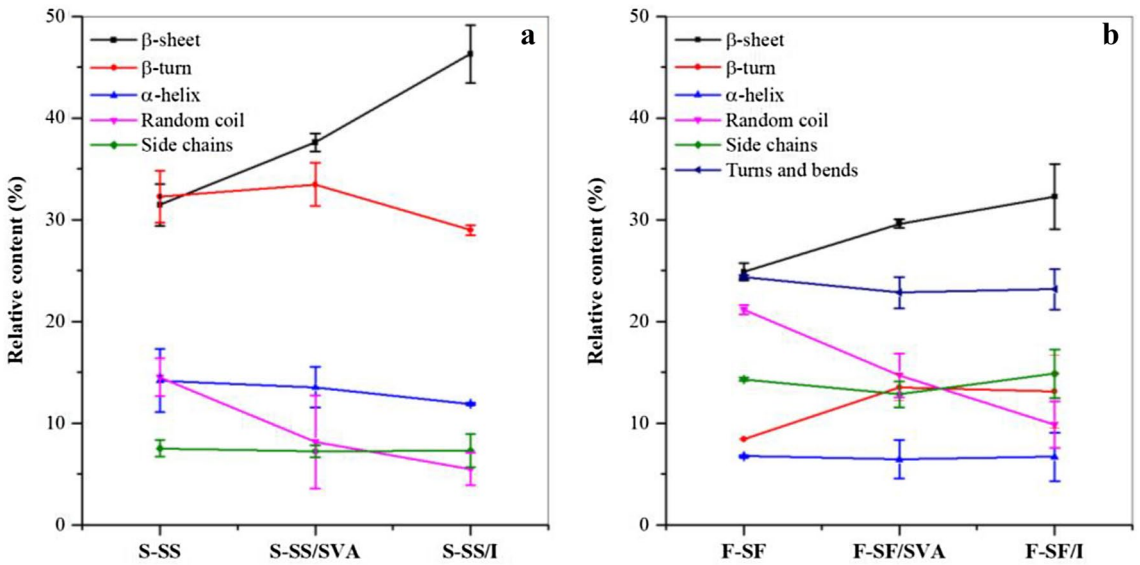

Fig. 2 Relative content of secondary structures in **a** S-SS and **b** F-SF materials

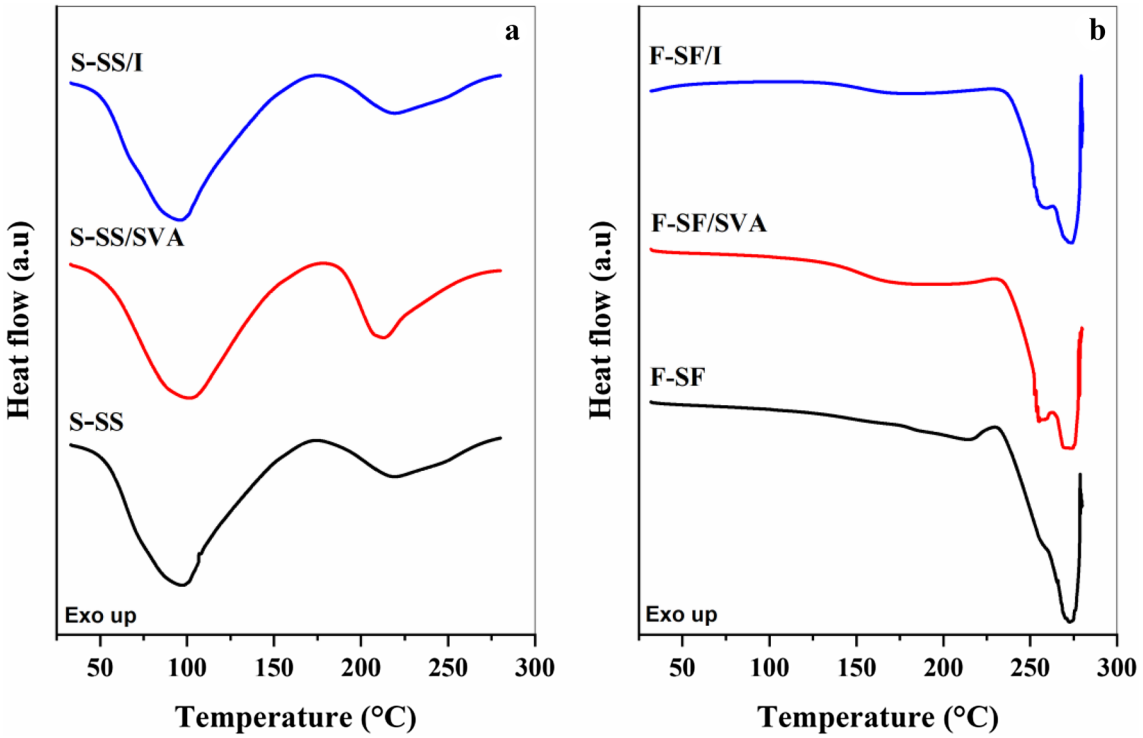

Fig. 3 DSC curves for **a** S-SS and **b** F-SF materials

3.3 Differential scanning calorimetry

Structural changes in SS and SF material after ethanol treatments were confirmed by DSC measurements (Fig. 3). In the case of S-SS, the treated and untreated samples have two endothermic peaks: the first one is due to the evaporation of bound water molecules, and the second one is attributed to the melt of the amorphous domains of the material [51–54]. From Fig. 3a, it is observed that S-SS/SVA sample presents this second event at lower temperature (212 °C) than that observed for S-SS/I (219 °C); hence, it can be concluded that immersion treatment favors more the crystalline phase compared.

In F-SF/I and F-SF/SVA DSC curves (Fig. 3b), the recrystallization peak that appears in the F-SF sample is present at 229.5 °C. This non-isothermal crystallization peak is related to the presence of amorphous secondary structures in the untreated samples that transform into β-sheets during DSC tests [55, 56]. Furthermore, the crystallization effect associated with the ethanol treatments produces protein samples with higher presence of crystalline secondary structures that obstructs chain movement and therefore the occurrence of crystallization by temperature. Finally, the data obtained by means of DSC are consistent with the relative increase in stable structures presented in the FTIR analysis.

4 Conclusions

From the results, it can be concluded that post-treatments with immersion and vapor of ethanol are effective to increase the content of the crystalline secondary structure, evidencing a transition from the amorphous structure random coil to the crystalline structure sheet-β. Immersion in ethanol provided the highest crystallinity for sericin sponges (75.28%) and fibroin films (43.14%) compared to vapor-treated and untreated materials. Similarly, the DSC results for treated sericin sponges and fibroin films are consistent with the relative increase in stable structures. On the other hand, the treated materials present morphological changes, which are more pronounced in the samples treated by I than by the SVA. Also, the immersion in ethanol produces more rounded pore geometry in S-SS/I and generates rough and fractured surface in F-SF/I. Finally, the influence of both post-treatments on the crystallinity and the morphology could be beneficial to improve the thermal and degradative stability of sericin and silk fibroin materials and properties which are important in cellular scaffolds.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Barajas-Gamboa JA, Alvarez-López C, Restrepo Osorio A, Serpa-Guerra AM (2016) Sericin applications: a globular silk protein. Ing Compet 18(2):193–206 **(ISSN 0123-3033)**
2. Joseph B, Raj SJ (2012) Therapeutic applications and properties of silk proteins from *Bombyx mori*. Front Life Sci 6(3–4):55–60. https://doi.org/10.1080/21553769.2012.760491
3. Teli MD, Rane VM (2011) Comparative study of the degumming of Mulberry, Muga, Tasar and Ericream silk. Fibres Text East Eur 85(2):10–14 **(ISSN 1230-3666)**
4. Gupta D, Agrawal A, Rangi A (2014) Extraction and characterization of silk sericin. Indian J Fibre Text Res 39(4):364–372 **(ISSN 09710426)**
5. Kunz RI, Brancalhão RMC, Ribeiro LDFC, Natali MRM (2016) Silkworm sericin: properties and biomedical applications. Biomed Res Int. https://doi.org/10.1155/2016/8175701
6. Kurioka A, Kurioka F, Yamazaki M (2004) Characterization of sericin powder prepared from citric acid-degraded sericin polypeptides of the silkworm, *Bombyx Mori*. Biosci Biotechnol Biochem 68(4):774–780. https://doi.org/10.1271/bbb.68.774
7. Zhang Y (2002) Application of natural silk sericin protein in biomaterials. Biotechnol Adv 20(2):91–100. https://doi.org/10.1016/S0734-9750(02)00003-4
8. Zhaorigetu S, Yanaka N, Sasaki M, Watanabe H, Kato N (2003) Inhibitory effects of silk protein, sericin on UVB-induced acute damage and tumor promotion by reducing oxidative stress in the skin of hairless mouse. J Photochem Photobiol B Biol 71(1–3):11–17. https://doi.org/10.1016/S1011-1344(03)00092-7
9. Kundu SC, Dash BC, Dash R, Kaplan DL (2008) Natural protective glue protein, sericin bioengineered by silkworms: potential for biomedical and biotechnological applications. Prog Polym Sci 33(10):998–1012. https://doi.org/10.1016/j.progpolymsci.2008.08.002
10. Vepari C, Kaplan DL (2007) Silk as a biomaterial. Prog Polym Sci 32(8–9):991–1007. https://doi.org/10.1016/j.progpolymsci.2007.05.013
11. Koh L-D et al (2015) Structures, mechanical properties and applications of silk fibroin materials. Prog Polym Sci 46:86–110. https://doi.org/10.1016/j.progpolymsci.2015.02.001
12. Dash BC, Mandal BB, Kundu SC (2009) Silk gland sericin protein membranes: fabrication and characterization for potential biotechnological applications. J Biotechnol 144(4):321–329. https://doi.org/10.1016/j.jbiotec.2009.09.019
13. Padamwar MN, Pawar AP, Daithankar AV, Mahadik KR (2005) Silk sericin as a moisturizer: an in vivo study. J Cosmet Dermatol 4(4):250–257. https://doi.org/10.1111/j.1473-2165.2005.00200.x
14. Wu JH, Wang Z, Xu SY (2007) Preparation and characterization of sericin powder extracted from silk industry wastewater. Food Chem 103(4):1255–1262. https://doi.org/10.1016/j.foodchem.2006.10.042
15. Nishida A, Yamada M, Kanazawa T, Takashima Y, Ouchi K, Okada H (2011) Sustained-release of protein from biodegradable sericin film, gel and sponge. Int J Pharm 407(1–2):44–52. https://doi.org/10.1016/j.ijpharm.2011.01.006
16. Teramoto H, Kameda T, Tamada Y (2008) Preparation of gel film from *Bombyx mori* silk sericin and its characterization as a wound dressing. Biosci Biotechnol Biochem 72(12):3189–3196. https://doi.org/10.1271/bbb.80375
17. Ang-atikarnkul P, Watthanaphanit A, Rujiravanit R (2014) Fabrication of cellulose nanofiber/chitin whisker/silk sericin bionanocomposite sponges and characterizations of their physical and biological properties. Compos Sci Technol 96:88–96. https://doi.org/10.1016/j.compscitech.2014.03.006
18. Kim SJ (2007) Gas permeation through water-swollen sericin/PVA membranes. Ms thesis, p 132. http://hdl.handle.net/10012/3098. Accessed 5 Jan 2019
19. Jaramillo-Quiceno N, Álvarez-López C, Restrepo-Osorio A (2017) Structural and thermal properties of silk fibroin films obtained from cocoon and waste silk fibers as raw materials. Procedia Eng 200:384–388. https://doi.org/10.1016/j.proeng.2017.07.054
20. Panico A, Paladini F, Pollini M (2019) Development of regenerative and flexible fibroin-based wound dressings. J Biomed Mater Res Part B Appl Biomater 107(1):7–18. https://doi.org/10.1002/jbm.b.34090
21. Bhardwaj N, Sow WT, Devi D, Ng KW, Mandal BB, Cho N-J (2015) Silk fibroin–keratin based 3D scaffolds as a dermal substitute for skin tissue engineering. Integr Biol 7(1):53–63. https://doi.org/10.1039/c4ib00208c
22. Bari E, Perteghella S, Faragò S, Torre ML (2018) Association of silk sericin and platelet lysate: premises for the formulation of wound healing active medications. Int J Biol Macromol 119:37–47. https://doi.org/10.1016/j.ijbiomac.2018.07.142
23. Aramwit P, Kanokpanont S, Nakpheng T, Srichana T (2010) The effect of sericin from various extraction methods on cell viability and collagen production. Int J Mol Sci 11(5):2200–2211. https://doi.org/10.3390/ijms11052200
24. Ko YG, Lee M, Park WH, Cho D, Kwon OK, Kwon OH (2016) Guiding bone regeneration using hydrophobized silk fibroin nanofiber membranes. Macromol Res 24(9):824–828. https://doi.org/10.1007/s13233-016-4109-2
25. Bonani W, Maniglio D, Motta A, Tan W, Migliaresi C (2011) Biohybrid nanofiber constructs with anisotropic biomechanical properties. J Biomed Mater Res Part B Appl Biomater 96(2):276–286. https://doi.org/10.1002/jbm.b.31763
26. Jeong L, Lee KY, Liu JW, Park WH (2006) Time-resolved structural investigation of regenerated silk fibroin nanofibers treated with solvent vapor. Int J Biol Macromol 38(2):140–144. https://doi.org/10.1016/j.ijbiomac.2006.02.009
27. Terada D, Yokoyama Y, Hattori S, Kobayashi H, Tamada Y (2016) The outermost surface properties of silk fibroin films reflect ethanol-treatment conditions used in biomaterial preparation. Mater Sci Eng C 58:119–126. https://doi.org/10.1016/j.msec.2015.07.041
28. Zhang H, Yang M, Min S, Feng Q, Gao X, Zhu L (2008) Preparation and characterization of a novel spongy hydrogel from aqueous *Bombyx mori* sericin. E-Polymers 066:1–10. https://doi.org/10.1515/epoly.2008.8.1.761
29. Kim MK, Kwak HW, Lee JY, Yun H, Kim MH, Lee KH (2012) Effect of lyoprotectant on the solubility and structure of silk sericin. Int J Ind Entomol 25(1):133–137. https://doi.org/10.7852/ijie.2012.25.1.133
30. Weska RF et al (2009) Effect of freezing methods on the properties of lyophilized porous silk fibroin membranes. Mater Res 12(2):233–237. https://doi.org/10.1590/S1516-14392009000200020
31. Rockwood DN, Preda RC, Yücel T, Wang X, Lovett ML, Kaplan DL (2011) Materials fabrication from *Bombyx mori* silk fibroin. Nat Protoc 6(10):1612–1631. https://doi.org/10.1038/nprot.2011.379
32. Siritientong T, Srichana T, Aramwit P (2011) The effect of sterilization methods on the physical properties of silk sericin scaffolds.

AAPS PharmSciTech 12(2):771–781. https://doi.org/10.1208/s12249-011-9641-y
33. Zhang K, Ye Q, Yan Z (2012) Influence of post-treatment with 75% (v/v) Ethanol vapor on the properties of SF/P (LLA-CL) nanofibrous scaffolds. Int J Mol Sci 13(2):2036–2047. https://doi.org/10.3390/ijms13022036
34. Siritienthong T, Ratanavaraporn J, Aramwit P (2012) Development of ethyl alcohol-precipitated silk sericin/polyvinyl alcohol scaffolds for accelerated healing of full-thickness wounds. Int J Pharm 439(1–2):175–186. https://doi.org/10.1016/j.ijpharm.2012.09.043
35. Teramoto H, Miyazawa M (2003) Analysis of structural properties and formation of sericin fiber by infrared spectroscopy. J Insect Biotechnol Sericol 72(3):157–162. https://doi.org/10.11416/jibs.72.157
36. De Chimie RR (2011) Rapid characterization of peptide secondary structure by FT-IR spectroscopy. Rev Roum Chim 56(8):783–791
37. Jackson M, Mantsch HH (1995) The use and misuse of FTIR spectroscopy in the determination of protein structure. Crit Rev Biochem Mol Biol 30(2):95–120. https://doi.org/10.3109/10409239509085140
38. Tao W, Li M, Xie R (2005) Preparation and structure of porous silk sericin materials. Macromol Mater Eng 290(3):188–194. https://doi.org/10.1002/mame.200400306
39. Chen X, Cai H, Ling S, Shao Z, Huang Y (2012) Conformation transition of *Bombyx mori* silk protein monitored by time-dependent fourier transform infrared (FT-IR) spectroscopy: effect of organic solvent. Appl Spectrosc 66(6):696–699. https://doi.org/10.1366/11-06551
40. Marelli B, Brenckle MA, Kaplan DL, Omenetto FG (2016) Silk fibroin as edible coating for perishable food preservation. Sci Rep 6:1–11. https://doi.org/10.1038/srep25263
41. Cai S, Singh BR (1999) Identification of β-turn and random coil amide III infrared bands for secondary structure estimation of proteins. Biophys Chem 80(1):7–20. https://doi.org/10.1016/S0301-4622(99)00060-5
42. Yang H, Yang S, Kong J, Dong A, Yu S (2015) Obtaining information about protein secondary structures in aqueous solution using Fourier transform IR spectroscopy. Nat Protoc 10(3):382–396. https://doi.org/10.1038/nprot.2015.024
43. Lawrence BD, Omenetto F, Chui K, Kaplan DL (2008) Processing methods to control silk fibroin film biomaterial features. J Mater Sci 43(21):6967–6985. https://doi.org/10.1007/s10853-008-2961-y
44. Jin H-J, Park J, Valluzzi R, Cebe P, Kaplan DL (2004) Engineered films of *Bombyx mori* silk with poly(ethylene oxide). Biomacromol 5(3):711–717. https://doi.org/10.1557/PROC-735-C11.11
45. Lamboni L, Gauthier M, Yang G, Wang Q (2015) Silk sericin: a versatile material for tissue engineering and drug delivery. Biotechnol Adv 33(8):1855–1867. https://doi.org/10.1016/j.biotechadv.2015.10.014
46. Chankow S (2016) Conformational transitions of thai silk fibroin secondary structures. In: Biomedical engineering international conference (BMEiCON-2016). https://doi.org/10.1109/BMEiCON.2016.7859597
47. Maghdouri-White Y, Bowlin GL, Lemmon CA, Dréau D (2016) Bioengineered silk scaffolds in 3D tissue modeling with focus on mammary tissues. Mater Sci Eng C 59:1168–1180. https://doi.org/10.1016/j.msec.2015.10.007
48. Yali W, Dan S, Honggen Y, Jiannan W (2014) Characterization of a PEG-DE cross-linked tubular silk scaffold. Text Res J 84(9):959–967. https://doi.org/10.1177/0040517513512401
49. Jo YN, Bae DG, Um IC (2013) The effect of extraction conditions and film side on the molecular conformation of silk sericin film. Int J Ind Entomol 26(2):113–118. https://doi.org/10.7852/ijie.2013.26.2.113
50. Yazawa K et al (2018) Combination of amorphous silk fiber spinning and postspinning crystallization for tough regenerated silk fibers. Biomacromolecules 19(6):2227–2237. https://doi.org/10.1021/acs.biomac.8b00232
51. Lungu A, Albu MG, Stancu IC, Florea NM, Vasile E, Iovu H (2013) Superporous collagen-sericin scaffolds. J Appl Polym Sci 127(3):2269–2279. https://doi.org/10.1002/app.37934
52. Mandal BB, Ghosh B, Kundu SC (2011) Non-mulberry silk sericin/poly (vinyl alcohol) hydrogel matrices for potential biotechnological applications. Int J Biol Macromol 49(2):125–133. https://doi.org/10.1016/j.ijbiomac.2011.03.015
53. Anghileri A, Lantto R, Kruus K, Arosio C, Freddi G (2007) Tyrosinase-catalyzed grafting of sericin peptides onto chitosan and production of protein-polysaccharide bioconjugates. J Biotechnol 127(3):508–519. https://doi.org/10.1016/j.jbiotec.2006.07.021
54. Oh H, Lee JY, Kim MK, Um IC, Lee KH (2011) Refining hot-water extracted silk sericin by ethanol-induced precipitation. Int J Biol Macromol 48(1):32–37. https://doi.org/10.1016/j.ijbiomac.2010.09.008
55. Xiao H, Kaplan D, Cebe P (2008) Dynamic protein – water relationships during β-sheet formation. Macromolecules 41(11):3939–3948. https://doi.org/10.1021/ma071551d
56. Lu Q et al (2010) Water-insoluble silk films with silk I structure. Acta Biomater 6(4):1380–1387. https://doi.org/10.1016/j.actbio.2009.10.041