

Preparation and Characterization of Silk Sericin/Glycerol/Graphene Oxide Nanocomposite Film

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(Received April 10, 2013; Revised June 26, 2013; Accepted July 9, 2013)

Abstract: Sericin (SS) is a protein that is secreted by silkworms, but it is usually discarded during the degumming process. To obtain and make use of the sericin, we prepared sericin/glycerol/graphene oxide nanocomposite film. The inherent brittleness of pure sericin film was improved by the addition of glycerol (Glc) as a plasticizer. To compensate for the reduced stiffness, we added graphene oxide (GO) into the SS/Glc film. At concentrations of up to 0.8 wt% relative to SS, GO dispersed evenly in the SS matrix without any agglomeration. The maximum tensile strength (9.5 ± 0.7 MPa) and Young's modulus (414.4 ± 23.2 MPa) were obtained when the GO content was 0.8 wt% relative to SS. The elongation of SS/Glc/GO nanocomposite film also increased by approximately 40 % compared to SS/Glc film. The strong interfacial interaction between the SS and the GO was responsible for the increased stiffness. The increased elongation was due to the reduced crystallinity of the sericin matrix in the presence of GO.

Keywords: Silk sericin, Graphene oxide, Nanocomposite, Plasticizer, Biocompostable material

Introduction

The development of the synthetic polymers has improved the quality of human life due to their adjustable properties for specific applications and low production costs. However, the future of the synthetic polymer is uncertain because of the exhaustion of petroleum resources and environmental concerns. As an alternative to synthetic polymers, those derived from nature are gaining attention. These bio-based polymers can be obtained directly as a polymer or synthesized using monomers that are produced from the biomass. Although the use of synthetic polymers currently overwhelms that of bio-based polymers, recent technological advances in bio-based polymers will lead to greater use of these polymers.

Sericin is a protein polymer that is secreted by the silkworm. Every year, approximately 400,000 tons of raw silk are produced worldwide; one-fourth of this raw silk is sericin. Unfortunately, despite its being available in huge quantities, the sericin is treated as a waste product of the degumming process. Therefore, much research has been performed to find an application for sericin [1,2]; however, the inherent drawbacks of sericin have made it impossible to use in the materials field. The biggest difficulty is the brittleness of sericin film. Various efforts have been made to overcome this problem, such as refining the sericin [3], blending the sericin with other polymers [4], grafting the sericin with synthetic polymers [5], or adding a plasticizer [6].

Most protein polymers, including sericin, require a plasticizer

to improve the processing of the polymer or to impart flexibility to the product. Various types of plasticizer have been added to protein polymers; most of these plasticizers were polyols, which possess multiple hydroxyl groups [7]. Glycerol is the most frequently used plasticizer due to its ease of penetration, good compatibility, and non-volatile properties [8]. However, the addition of a plasticizer sacrifices the tensile properties of the material. The role of the plasticizer is to increase the free volume of the polymer chain, but as a result, the polymer chain packing is loosened, resulting in the loss of chain integrity. Previously, Zhang et al. reported that the addition of glycerol improved the elongation of sericin film from 0.73 to 354 %, but decreased the tensile strength from 13.72 to 8.19 MPa [6]. A decrease in tensile strength would be unacceptable if the tensile strength drops below some specific level that is required for the use of the material.

Nanocomposites are composite materials made of nano-substances, such as nanoclays, nanoparticles, nanofibers, carbon nanotube, and others, as filler material. Recently, graphene, graphene oxide (GO), and reduced graphene oxide have been used as a filler material. The addition of the graphene-family nanomaterials greatly enhances the mechanical properties of a material once the filler material has been evenly distributed. Among the graphene-family nanomaterials, GO appears the most suitable for nanocomposite because it has less of a tendency to agglomerate itself compared to pristine graphene, and a wide range of functionalities could be introduced [9,10]. Various types of polymers have been researched for the preparation of nanocomposite material

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with GO, including polypropylene [11], polyvinyl alcohol [12], poly(3-hydroxybutyrate-co-4-hydroxybutyrate) [13], alginate [14], carboxymethyl cellulose [15], chitosan [16], starch [17], and others.

The objective of this study is to prepare a flexible sericin film with increased tensile strength. We introduced glycerol to improve the flexibility of sericin film. Furthermore, to compensate for the reduction in tensile strength, we added GO as a nanofiller material. The mechanical properties and structure of the sericin/glycerol/graphene oxide (SS/Glc/GO) nanocomposite film were then evaluated.

Materials and Methods

Materials

Silk cocoons were kindly given by the Heungjin Co. (Yongin, Korea). Graphite was purchased from Fisher Scientific (Leics, UK). All of the other chemicals were purchased from Sigma-Aldrich (Yongin, Korea).

Sericin Extraction

Silk cocoons were boiled in an autoclave at 120 °C for 1 hr. The liquor ratio was 1:25. After extraction, the remaining cocoons were removed from the sericin solution, and the solution was filtered with a nonwoven filter paper. The final concentration of sericin solution was 1.0 wt%.

Preparation of GO

GO was prepared according to a modified Hummer method. Graphite powder was added to an 80 °C solution of H₂SO₄, K₂S₂O₈, and P₂O₅. The resulting mixture was maintained at 80 °C for 6 hours and cooled to room temperature, diluted, and washed with distilled water. Then, the mixture was filtered to remove the residual acid and air-dried at room temperature overnight. This pre-oxidized graphite was oxidized by Hummer's Method. The pre-treated graphite powder was added to cold (0 °C) concentrated H₂SO₄. KMnO₄ was added gradually with stirring, and the temperature of the mixture was maintained at less than 20 °C during the procedure. The mixture was then stirred at 35 °C for 2 hrs, and dH₂O was added. Lastly, a large amount of dH₂O and 30 % H₂O₂ solution was added to the mixture to terminate the reaction. The resulting solution was filtered and washed with 1:10 HCl solution to remove metal ions. The GO slurry was purified by dialysis for 3 days and was dried in a 50 °C dry oven.

Preparation of SS/Glc/GO Nanocomposite Film

Glycerol was added to the sericin solution as a plasticizer. Typically, the sericin solution was concentrated with a rotary evaporator until the concentration of sericin reached 2 wt%. To 50 ml of sericin solution, 0.6 g of glycerol (Glc) was added. The SS/Glc film was prepared by casting this solution at 50 °C in an oven for one day. The SS/Glc/GO nanocomposite films were prepared by adding GO to the SS/Glc

solution. Specifically, the GO was first dispersed in water and sonicated for 1 hour. The GO suspension was added to the SS/Glc solution and the amount was adjusted to a final GO concentration of 0.4, 0.8 and 1.2 % relative to sericin. The SS/Glc/GO solution was cast in a 50 °C dry oven for one day. The prepared nanocomposite films were labeled according to the content of GO relative to sericin, as SS/Glc/GO04, SS/Glc/GO08 and SS/Glc/GO12.

Mechanical Characterization

The mechanical properties of SS/Glc and SS/Glc/GO nanocomposite films were measured by a Universal testing machine (UTM, LRX plus, LLOYD instruments, UK). All of the samples were formed into strips of 50×5×0.1 mm. The tensile test was performed according to ASTM D 638 (25.4 mm/min) at room temperature. At least ten samples were tested for each type of nanocomposite film.

Morphological and Structural Characterizations of GO and SS/Glc/GO Nanocomposite Films

The morphology of GO as prepared by this method was investigated using transmission electron microscopy (TEM, JEM1010, JEOL, Japan). The difference between the crystallographic structure of the graphite and that of GO was determined using a powder X-ray diffraction system (MiniFlex600, USA). Various functional groups in the GO were investigated using attenuated total reflectance-Fourier transform infrared spectrometer (ATR-FTIR, Nicolet 6700, Thermo Scientific, USA).

A field emission scanning electron microscope (FE-SEM, JSM-7600F, JEOL, Japan) was employed to observe a cross-section of the SS/Glc/GO nanocomposite films. The crystallinity of the SS/Glc/GO nanocomposite films was determined using a wide-angle X-ray diffraction system (WAXS, Bruker, Germany) equipped with CuK α radiation.

The secondary structure of the SS/Glc/GO nanocomposite films was measured using an ATR-FTIR spectrometer. The secondary structure content was measured as in previous studies. The secondary structure composition of the SS/Glc/GO nanocomposite film was determined at the amide I band between 1600 and 1700 cm⁻¹ through the peak-fitting method. The second derivative spectra for calculating the number and position of the overlapped single bands and the deconvolution spectra for fitting were obtained using Origin 8.0 software. The curve-fitting proofs were performed until Chi²/Dof reached the minimum value with the multi-peak Gaussian fitting. The relative area of the overlapped single bands was used for estimating the proportion of secondary structures, such as random coils, α -helices, β -sheets and turns. The single bands from the nanocomposite films were assigned to corresponding secondary structures, according to previous studies [6]: 1605-1615 cm⁻¹, aggregated strands; 1616-1637 cm⁻¹, β -sheet; 1638-1655 cm⁻¹, random coil; 1656-1662 cm⁻¹, α -helices; 1663-1695 cm⁻¹, turns.

Results and Discussion

Preparation of GO

Figure 1(a) shows a TEM image of the final GO prepared by the modified Hummer's method. The graphite is exfoliated into individual sheets, and this result can also be observed in the XRD diffractogram shown in Figure 1(b). Graphite exhibits a characteristic peak at $2\theta=26^\circ$, but this peak disappears when the GO is formed. At this point, a new peak is observed at approximately $2\theta=11^\circ$, which is a characteristic peak of GO. The ATR-FTIR spectrum in Figure 1(c) also reveals the formation of GO, as functional groups such as -OH (3400 cm^{-1}), carboxyl -C=O and -C-O (1720 and 1415 cm^{-1}), aromatic -C=C (1620 cm^{-1}), epoxy -C-O (1280 cm^{-1}) and alkoxy -C-O (1080 cm^{-1}) can be found [18]. These results indicate that we successfully prepared exfoliated GO by the modified Hummer's method.

Mechanical Properties of the SS/Glc/GO Nanocomposite Films

The inherent brittleness of pure sericin film must be improved for it to be used as a material. When glycerol is added as a plasticizer, the elongation of the sericin film is greatly enhanced, but the tensile strength decreases [6]. In most cases, the addition of the plasticizer would be beneficial only if the decreased tensile strength is still high enough for the film to function. However, sericin film itself is not strong enough when compared to other polymers; therefore, the extensibility of the sericin film must be enhanced without the loss of tensile strength when the plasticizer is added. In this study, we added GO to the SS/Glc flexible film as a

filler to compensate for the decrease in or even to improve the tensile strength.

Figure 2 demonstrates the tensile strength, Young's modulus and elongation at break of the SS/Glc and SS/Glc/GO films. The pure sericin film was too brittle even to fit in the grip of the tester. However, when 0.6 g of glycerol was added to 1 g of sericin, the film became flexible and we were able to measure the mechanical properties. The elongation at break of the SS/Glc film was $29.5\pm 6.7\%$, and the tensile strength and Young's modulus of this film were $9.5\pm 0.7\text{ MPa}$ and $414.4\pm 23.2\text{ MPa}$, respectively. When the GO was added to the SS/Glc film, both the tensile strength and Young's modulus increased. The maximum tensile strength and Young's modulus were obtained with the SS/Glc/GO08 film and were determined to be $13.7\pm 1.1\text{ MPa}$ and $554.2\pm 30.8\text{ MPa}$, respectively. However, in the case of the SS/Glc/GO12 film, the tensile strength and Young's modulus decreased to $11.9\pm 2.0\text{ MPa}$ and $495\pm 69.5\text{ MPa}$, respectively. The elongation at break of the SS/Glc/GO films was higher than that of the SS/Glc film, except for the SS/Glc/GO12 film. In the case of the SS/Glc/GO08 film, the elongation at break was $41.4\pm 13.1\%$ which increased by 40 % compared to the SS/Glc film.

The SS/Glc/GO12 film exhibited the lowest tensile strength, Young's modulus and elongation among the SS/Glc/GO films. The synergistic effect of nanocomposite occurs only when the nanofiller is well dispersed in the matrix. Figure 3 shows the SEM images of the cross-section of the SS/Glc and SS/Glc/GO films. While the SS/Glc/GO04 and SS/Glc/GO08 did not show any agglomerated GO structure, a quantity of agglomerated GO could be observed in the case of the SS/Glc/GO12 film. The agglomeration of GO is more clearly

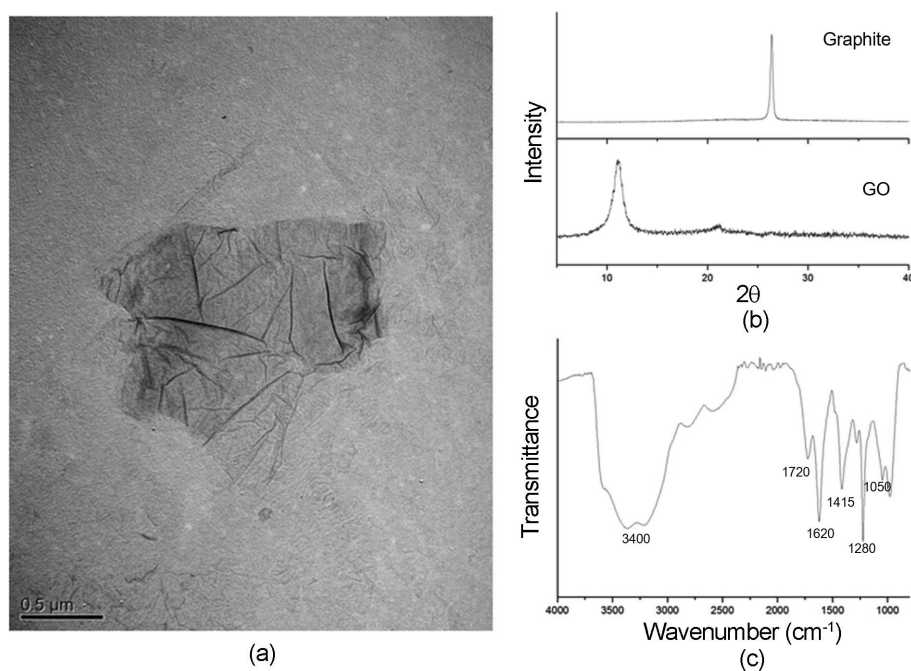


Figure 1. TEM image (a), XRD diffractogram (b), and FT-IR spectrum (c) of GO used in this study.

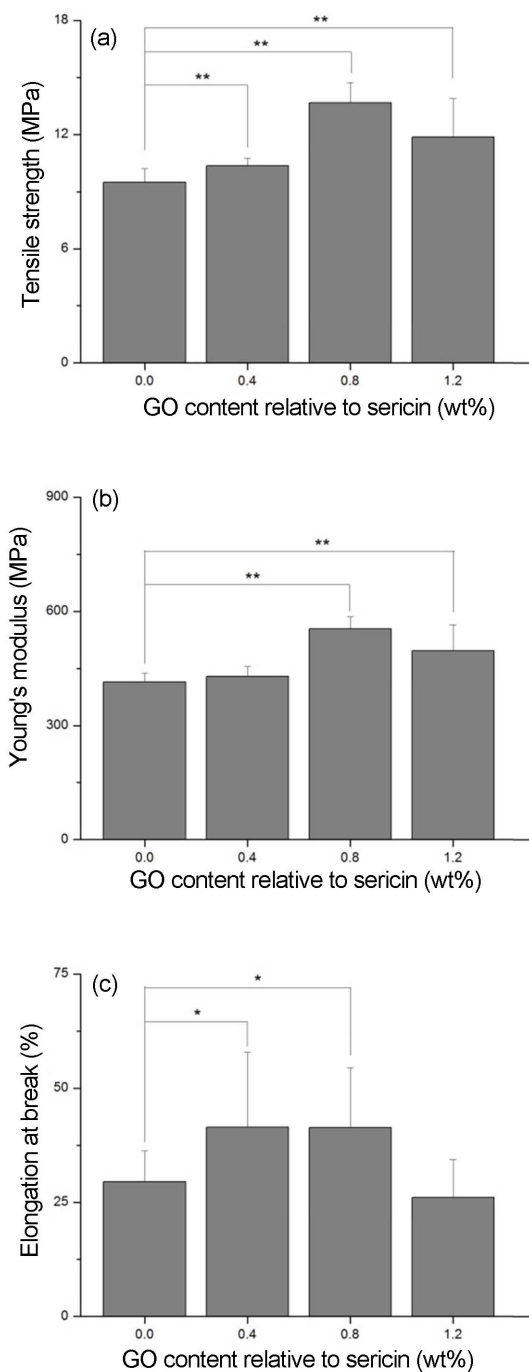


Figure 2. Mechanical properties of SS/Glc and SS/Glc/GO nanocomposite films; (a) tensile strength, (b) Young's modulus, and (c) elongation at break. * and ** indicate significant differences of $p < 0.05$ and $p < 0.01$, respectively, as determined using Student's *t*-test.

observed when the content of GO was 1.6 wt% of sericin (Figure 3(e)). Therefore, the poor mechanical properties of SS/Glc/GO12 film are due to the agglomeration of GO.

The increase in the tensile strength and Young's modulus

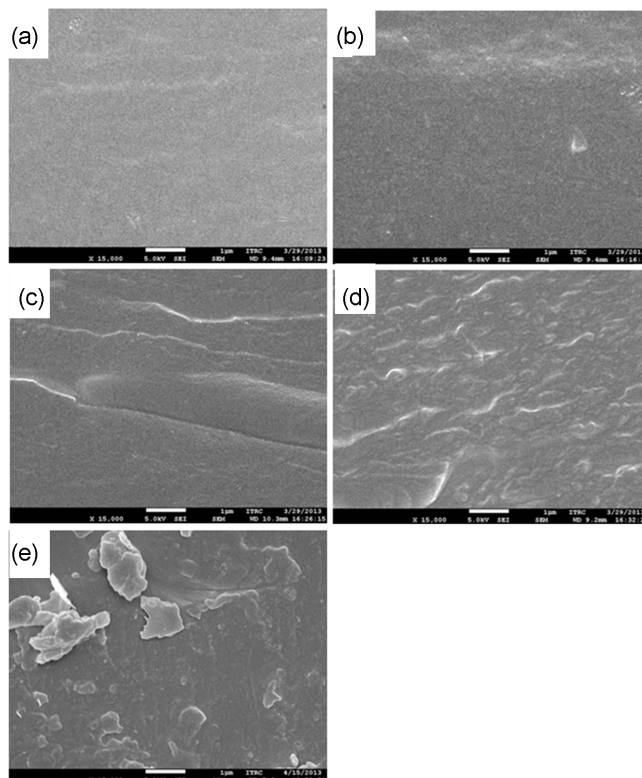


Figure 3. SEM images of cross-sections of the SS/Glc and SS/Glc/GO nanocomposite films; (a) SS/Glc, (b) SS/Glc/GO04, (c) SS/Glc/GO08, (d) SS/Glc/GO12, and (e) SS/Glc/GO16. In the case of SS/Glc/GO16, the GO content is 1.6 wt% relative to sericin.

of the SS/Glc/GO04 and SS/Glc/GO08 films can be explained by the conventional composite theory. A good interfacial adhesion between the matrix and the filler increases the tensile strength and the Young's modulus of a composite film. GO has epoxy, carboxyl and hydroxyl groups that can form electrostatic interactions or can hydrogen bond with the sericin. The pH of the sericin solution is approximately 6, and pI of sericin is approximately 4 [19]; therefore, the net charge of the sericin would be negative at pH 6. However, the amino groups of sericin remain positively charged because their pKa value is higher than 9. Because the carboxyl groups of GO are negatively charged at pH 6, electrostatic interactions occur between the amino groups of sericin and the carboxylate groups of GO. Pan *et al.* [16] explained the strengthening effect of GO on chitosan film by the formation of hydrogen bonds between the epoxy, carboxyl and hydroxyl groups of GO and the amino and hydroxyl groups of chitosan. The same interaction between sericin and GO also possible because sericin also possesses various functional groups, such as hydroxyl, carboxyl and amino groups, which are capable of forming hydrogen bonds with the functional groups of GO.

Interestingly, with the addition of GO up to 0.8 % of wt

relative to sericin, the elongation property of the SS/Glc/GO film is also improved. This is contrary to previous reports, in which the elongation of a nanocomposite film decreases in the presence of GO [12-15]. In these previous studies, the elongation of GO nanocomposite film declines because the GO constrains the mobility of the polymer molecules. However, in certain other cases, a simultaneous increase in both tensile strength and elongation has been observed [16, 17,20,21]. To elucidate this result, we performed XRD and FT-IR analysis on the well-dispersed SS/Glc/GO films, observing the effect of GO on the crystallinity and secondary structure of sericin.

Effect of GO on the Crystallinity and Secondary Structure of Sericin

To our best knowledge, there is no agreement as to why both tensile strength and elongation increase in nanocomposite materials. Surprisingly, this outcome has been found most commonly in nanocomposite films where the matrix polymer has many functional groups for hydrogen bonding with GO; for example, chitosan [16,20,21] and starch [17]. The strong hydrogen bonding leads to strong interfacial interactions between the matrix (chitosan, starch or sericin) and the filler (GO). Due to these strong interfacial interactions, load transfer from the matrix to the GO might be facilitated [10,16,20], and the toughness is thereby increased. In addition, Ma *et al.* [17] speculated that starch can slip at the surface of a GO sheet.

Another possible cause of the increased elongation is the crystallinity change of the SS/Glc film, due to the addition of GO. We performed XRD analysis to determine whether the addition of GO affects the crystallinity of the SS/Glc film. Figure 4 shows the XRD diffractogram of the sericin, SS/Glc and SS/Glc/GO films. In the presence of Glc, the β -sheet structure of sericin is more developed [6]; therefore,

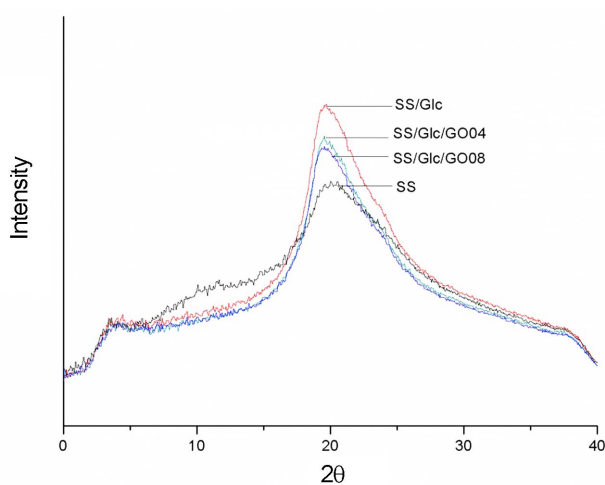


Figure 4. XRD diffractograms of SS, SS/Glc and well-dispersed SS/Glc/GO nanocomposite films.

the crystallinity of the SS/Glc film has increased compared to the sericin film. When GO is introduced, however, the crystallinity of the nanocomposite films decreases. This decrease in crystallinity could be why the elongation increases in the presence of GO. The strong interactions between the sericin and GO might prevent the crystallization of sericin by constraining the mobility of the sericin chain. However, we must consider that the tensile strength and Young's modulus increases with the addition of GO, which is contrary to the XRD results. Thus, we can summarize the role of GO in the sericin matrix as follows: either reinforcing the sericin matrix or reducing the crystallinity of the sericin matrix. If the addition of GO does not contribute to the reinforcement of the sericin matrix, but rather prevents the crystallization of the sericin matrix, then the tensile strength and Young's modulus should be decreased. However, the addition of GO dissipates the load by load transfer due to strong interfacial interactions between sericin and GO, and the actual load placed on the sericin matrix would be far lower than would be necessary to cause the failure of the sericin matrix. As a result, sericin matrix can be extended much more than expected. In the case of the SS/Glc/GO04 film, the increase in the percent strain at break occurs without a significant increase in the tensile strength or Young's modulus compared to the SS/Glc film. This result indicates that the reduction in crystallinity was compensated for by the reinforcing effect of GO. In the case of the SS/Glc/GO08 film, to which more GO is added, the effect of the reinforcement exceeds the effect of reducing crystallization; therefore, the elongation remains unchanged, while the tensile strength and Young's modulus increase.

We further examined how the secondary structure of sericin is affected in the presence of GO. Figure 5 shows the

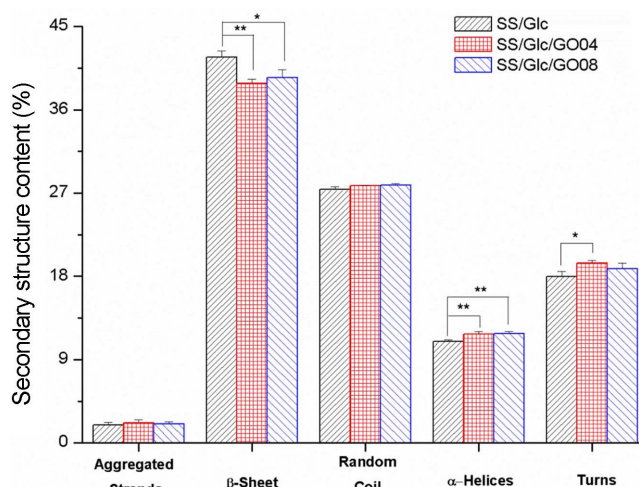


Figure 5. The content of the secondary structure of the sericin matrix in the SS/Glc and well-dispersed SS/Glc/GO nanocomposite films. * and ** indicate significant differences of $p < 0.05$ and $p < 0.01$, respectively, as determined using Student's *t*-test.

content of each type of secondary structure in sericin in the presence of Glc and the presence of both Glc and GO. The unusually high content of β -sheet structure that is observed is due to the presence of Glc, which is in agreement with the results of a former study [6]. Through the addition of GO, the amount of β -sheet structure has decreased, while that of random coils, α -helices and β -turn structure has increased slightly compared to the SS/Glc film. As crystallinity is closely related to β -sheet structure, the result is coincident with the XRD data. It therefore could be concluded that the increased elongation observed for the SS/Glc/GO film is due to the reduced crystallinity of the SS matrix. Regardless, the toughness of the SS/Glc film, which corresponds to the area under the curve of the stress-strain curve, has been increased by the addition of GO up to 0.8 wt%.

Conclusion

In this study, we have prepared SS/Glc/GO nanocomposite film to improve the mechanical properties of sericin film. The nanocomposite film exhibits increased tensile strength, Young's modulus and elongation at break when GO is added at a concentration of 0.8 wt% relative to sericin. The strong interfacial interactions between sericin and GO contribute to the increased tensile strength and Young's modulus. The elongation was increased due to the reduced crystallinity of the sericin matrix in the presence of GO. We expect that the sericin nanocomposite film could be used as a biocompostable material in various fields.

Acknowledgments

This work was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Education (NRF-2010-0025378).

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