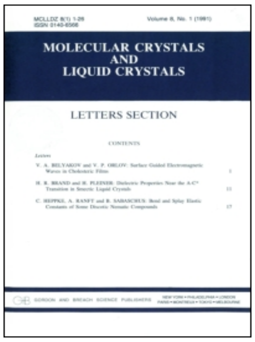

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Polymer films based on silk fibroin and collagen - the physico-chemical properties

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ABSTRACT
Thin films based on the blend of collagen and silk fibroin were prepared. Biopolymers were dissolved separately in an aqueous solution, mixed together, and after solvent evaporation films were obtained. The surface properties of films have been studied by the contact angle measurements and atomic force microscopy. The mechanical properties such as Young modulus, tensile strength and elongation at break were measured. The results suggest that modification of properties of biopolymeric materials is a consequence of the strong interaction between the polymeric components. The structure and surface properties of two-component blends depend on the mixture composition.

KEYWORDS
collagen; silk fibroin; biomaterials; blends; films

1. Introduction

Materials based on natural polymers are widely applied in biomedical field due to their variety of properties [1]. Special attention has been paid on the blends of two or more natural polymers, because using the blend a new material with a better properties than the single substance can be obtained [2]. Composite materials composed of two proteins: collagen (Col) and silk fibroin (SF) may exhibit an effective improvement of the characteristics [3]. It can be interesting starting material for developing several forms, e.g., films, sponges, foams or hydrogels [3,4].

The major producer of silk throughout the world is mulberry silkworm (*Bombyx mori*) [5]. The raw silk fiber forming a cocoon, is composed of two protein portions: fibroin (75–83%), which is a core of the fiber and sericin (17–25%), acting as the glue [6,7]. Sericin is capable of causing allergic and immunologic reactions in humans, therefore silk fibers must first undergo the degumming process through a boiling in water, soap or acid treatments [6,8–10]. Silk fibroin (SF) is mainly made of repeatable sequence of amino acids, such as glycine (43%), alanine (30%), and serine (12%) [11,12]. SF can arrange itself into two structural conformation: silk I (α-helix) and silk II (β-sheets) [12]. Silk I can be soluble in water, while silk II can be only dissolved using aqueous inorganic salt (e.g., LiBr, LiSCN) or concentrated acids, because those solutions have ability to break hydrogen bonds stabilizing a β-sheets structure [13–15]. SF is very interesting materials for use either in biomedical or cosmetics fields, due to its favorable properties. Namely, it has controllable biodegradability, excellent biocompatibility, both

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in vivo and *in vitro*, and low antigenicity and unique mechanical properties. Moreover, it has the ability to oxygen transmissibility [16,17]. Recently, its antioxidant properties have been proven [7]. Despite the fact, that native SF fibers are mechanically very stable, a most of the regenerative SF materials are rather brittle and weak [13].

Collagen (Col) type I can be obtained from different animal tissues, e.g.: the skin and bone of cow, skin and scales of fish or rat tail tendon [18]. This fibrous protein is mainly composed of a repetitious sequence of four amino acids: glycine, proline, hydroxyproline and alanine [19,20]. Collagen type I forms a characteristic triple helix structure [18]. Due to the presence of several crosslinks within the molecule it is mostly insoluble in water [1]. Collagen, similarly as SF, displays excellent biocompatibility, biodegradability and it is not immunogenic. Moreover, it has a good homeostatic properties and biofunctionalities that cells can recognize this protein [3,11,23]. Owing to these properties, collagen-based biomaterials are very important for tissue engineering and regenerative medicine [22].

In the literature there are only a few reports about the physico-chemical properties of the films based on the blend of collagen and silk fibroin [23,24]. Thus, the surface and mechanical properties of collagen/silk fibroin blends become the main focus on this work. Our previous study of silk fibroin composites modified by collagen showed that these two polymers are miscible and sponges made of silk fibroin/collagen mixture can be interesting materials for tissue engineering [25]. The main aim of the present study was to prepare Col/SF blend films in different weight ratios by a solvent evaporation technique. The obtained materials were characterized using AFM technique, contact angle measurements and tensile tests was performed.

2. Materials and methods

2.1. Preparation of silk fibroin and collagen mixtures

Collagen was obtained in our laboratory from tail tendons of young rats. Collagen fibers from rat tendons were washed in distilled water and dissolved in 0.1M acetic acid. The obtained solution was then centrifuged and the soluble fractions were decanted. Next, soluble collagen was lyophilized [26]. Silk fibroin was extracted from *B. mori* cocoons (Jedwab Polski Sp. z o.o company) also in our laboratory. Cocoons were boiled for 1 h in aqueous solution of 0.5% Na_2CO_3 twice, according to the procedure found in literature [25] and then cocoons were boiled in 5% alkaline soap solution for 30 minutes and then for 20 minutes in distilled water to extract the sericin proteins [9]. This procedure was repeated three times. The degummed silk was dissolved in a $CaCl_2/H_2O/CH_3CH_2OH$ (molar ratio: 1:8:2) at 80°C for 4 h. Then the fibroin solution was filtered and dialyzed against distilled water for 3 days to yield a fibroin aqueous solution [27]. The final fibroin concentration was 1% (it was determined by weighing the remaining residue after drying). Collagen solution in 0.1M acetic acid was prepared in concentration 1% wt. Solutions of these two polymers were mixed in weight ratios of silk fibroin to collagen: 90/10, 80/20, 70/30, 60/40, 50/50, 40/60, 30/70, 20/80, 10/90. Polymer films were obtained by casting the mixture of two polymers (in different compositions) in solution onto a glass plate. The film samples were dried in the vacuum oven at room temperature.

2.2. Contact angle measurements

The measurements of contact angles (Θ) of two liquids: diiodomethane (D) and glycerol (G) on the surface of collagen, silk fibroin and collagen/silk fibroin films were measured at room temperature using the DSA10 goniometer of Krüss GmbH (Germany), equipped with software for the drop shape analysis. The liquid drop of 2–3 μl was placed onto the polymer surface with a microsyringe. The drop image was recorded by video camera and digitalized. The drop shape was solved numerically and fitted by means of the mathematical functions using instrument software. Each value of contact angle is an average of 5 measurements, whose precision was 0.2°.

2.3. Mechanical properties

Mechanical properties of the materials were measured by a mechanical testing machine Z.05, Zwick/Roell, Germany. Mechanical properties (Young Modulus, breaking force and elongation at break) of collagen with different additions of silk fibroin were measured at room temperature in the dry state and at a crosshead speed of 5 mm/min in accordance with the Polish Norm PN-81/C-89034 (ISO 527-1 i 527-2) standard procedure. Samples were cut with a sharper of initial dimensions 54 mm length, 10.06 mm width and 20–25 μm thickness. The thickness of the sample was measured by an ultrameter type A-91 (producer: Manufacture of Electronic Devices, Warsaw, Poland). All the samples of film were cut using the same shaper. For each kind of film, at least five samples were tested.

2.4. Atomic force microscopy (AFM)

Topographic images were obtained using a multimode scanning probe microscope with a NanoScope IIIa controller (Digital Instruments, Santa Barbara, CA) operating in the tapping mode, in air, at room temperature. Surface images were acquired at fixed resolution (512 × 512 data points) using scan width 5 μm with a scan rate of 1.97 Hz. Silicon tips with spring constant 2–10 N/m were used. Roughness parameter such as the root mean square (R_q) was calculated from 5 μm × 5 μm scanned area using Nanoscope software.

3. Results and discussion

3.1. Contact angle measurements

For the films obtained from the blend of collagen and silk fibroin, the surface properties such as the wettability and roughness were measured. Using the contact angle measurements the values of surface free energy were calculated by the Owen-Wendt equation [28]. Table 1 gives the values of contact angles, surface free energy and its polar and dispersive components for the collagen and silk fibroin samples and their blends. As it can be seen, the surfaces of pure polymer films and their blends were rather hydrophobic as the values of glycerol contact angles were higher than the values of diiodomethane contact angles. The lowest value of glycerol contact angles was found for the collagen and Col/SF blend with 90% of collagen, which suggests that the surface of the blend with this composition is the most hydrophilic. The values of surface free energy as well as dispersive and polar components indicate that the collagen film and the blend film with 90% and 50% of collagen have the highest polarity among the selected specimens because they have the highest polar component of surface free energy. In the case of silk fibroin and Col/SF blends, the values of dispersive component are much higher than these of polar component which indicates mainly hydrophobic properties of the surface. This behavior indicates some interactions between collagen and silk fibroin in the polymer blend. Therefore, the polar reactive groups of polymers are being hidden below the film surface.

Table 1. Values of contact angle [deg], surface free energy [γ_S] and its polar [γ_S^p] and dispersive [γ_S^d] components for Col, SF and their blends.

	Contact angle [○]					
%Col	D	G	γ_S[mJ/m^2]	$\gamma_S{}^d$[mJ/m^2]	$\gamma_S{}^P$[mJ/m^2]	$\gamma_S{}^P/\gamma_S{}^D$
0	51.4 ± 0.3	88.7 ± 0.4	33.13	32.29	0.84	0.026
10	50.8 ± 0.1	89.4 ± 0.2	33.55	32.88	0.67	0.02
20	50.4 ± 0.1	86.4 ± 0.2	33.56	32.33	1.23	0.038
30	49.6 ± 0.1	85.0 ± 0.3	33.97	32.49	1.48	0.046
40	50.1 ± 0.8	84.3 ± 0.9	33.68	31.97	1.71	0.053
50	49.9 ± 1.8	82.4 ± 0.7	33.82	31.59	2.23	0.071
60	47.6 ± 0.2	83.5 ± 0.2	35.05	33.39	1.66	0.050
70	48.0 ± 0.2	83.6 ± 0.3	34.83	33.15	1.67	0.050
80	45.3 ± 0.5	82.9 ± 0.5	36.28	34.70	1.58	0.046
90	44.3 ± 0.8	80.9 ± 0.3	36.80	34.78	2.02	0.058
100	44.4 ± 0.2	80.4 ± 0.4	36.76	34.58	2.18	0.063

Note: G – glycerol, D – diiodomethane, $\%_{Col}$ – percentage fraction of collagen

3.2. Mechanical properties

The characteristic stress-strain curves of collagen and silk fibroin films and films made of Col/SF were obtained by Zwick&Roell 0.5 testing machine. Stress-strain curves for collagen, silk fibroin films and films made of Coll/SF blends show similar shape (curves not shown). Young's modulus of pure polymer films and films made of Col/SF blends is shown in Figure 1. Table 2 gives the corresponding values of tensile strength, Young's modulus and the elongation at break. It can be noticed that the Col/SF blend films exhibit higher values of tensile strength and Young's modulus than the silk fibroin film (Figure 1 and Table 2). The addition of collagen into silk fibroin is shown here to have an effect on all the mechanical parameters of the films measured in this study.

The tensile strength and Young's modulus of silk fibroin film increases after mixing SF with collagen. It may suggest that during the mixing of silk fibroin with collagen the new crosslinking reactions in the blend film responsible for increase of mechanical properties occur. The

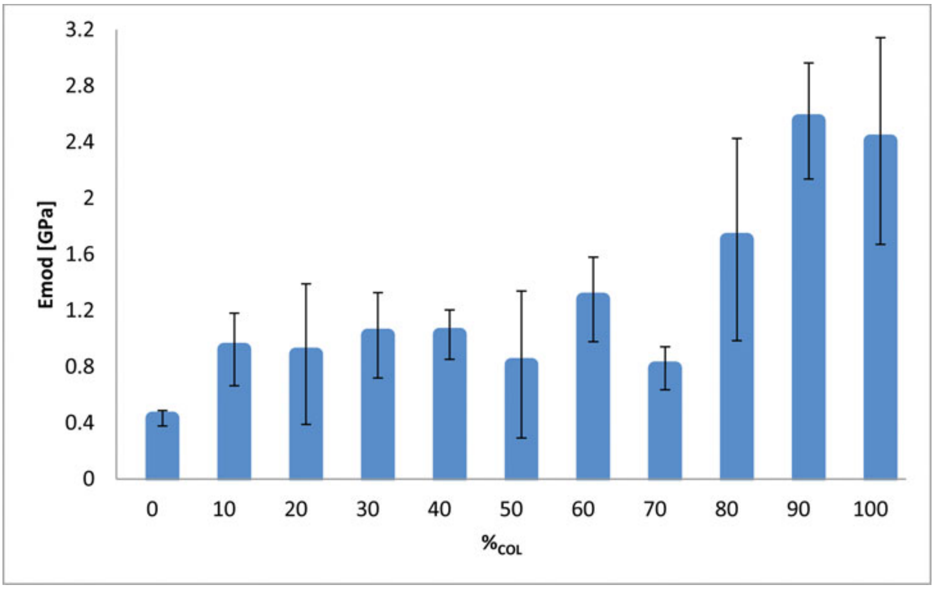

Figure 1. Young's modulus of Col, SF and their blends.

Table 2. Parameters of mechanical properties of Col, SF and their blends.

$\%_{Col}$	E_{mod} [GPa]	F_{max} [MPa]	dl [%]
0	0.43 ± 0.06	4.12 ± 0.94	0.63 ± 0.04
10	0.92 ± 0.26	4.99 ± 1.34	0.63 ± 0.11
20	0.89 ± 0.50	26.3 ± 16.6	0.83 ± 0.24
30	1.02 ± 0.30	9.63 ± 2.51	0.67 ± 0.13
40	1.03 ± 0.18	35.2 ± 9.30	1.27 ± 0.29
50	0.82 ± 0.52	21.9 ± 10.7	0.73 ± 0.18
60	1.28 ± 0.30	20.6 ± 9.6	0.93 ± 0.22
70	0.79 ± 0.15	1.70 ± 1.06	0.30 ± 0.07
80	1.71 ± 0.72	3.04 ± 1.01	1.30 ± 0.33
90	2.55 ± 0.41	66.1 ± 4.93	5.10 ± 0.13
100	2.41 ± 0.74	81.2 ± 1.87	0.96 ± 0.29

Note: $\%_{Col}$ – percentage fraction of collagen, E_{mod} - Young's modulus, F_{max} - tensile strength, dl - the elongation at break

presence of intermolecular interactions was confirmed in our previous study which has been observed by the viscometric method and FTIR spectra [25]. In the case of the elongation at break (Table 2), the value for silk fibroin was not significantly changed by the addition of collagen. The exception is the Col/SF blend film with 90% of collagen where there is clear increase in the value of the elongation at break. This may indicate strong interactions between polymers in this blend.

3.3. Atomic force microscopy

Structural changes on the surface of collagen, silk fibroin and collagen containing different additions of silk fibroin films have been observed using AFM microscope. The examples of AFM images of pure collagen, silk fibroin and Col/SF blend films are shown in Figures 2–3 and the roughness parameters are shown in Table 3. The AFM images show the difference in the surface properties of films for the pure polymers and their blends. The surface morphology of pure polymer films is considerably rough, which can results from the crystalline of the sample. In the case of Col/SF blends, the surface morphology of blend depends on its composition. For the Col/SF blends with 20% and 90% of collagen (Figures 3B and 3I), the surface of blend is significantly more folded than the surface of pure polymer and other composition films. This may indicate an increase in the heterogeneity of this blend in comparison to other blend films. In the case of the Col/SF blend with the small addition of silk fibroin,

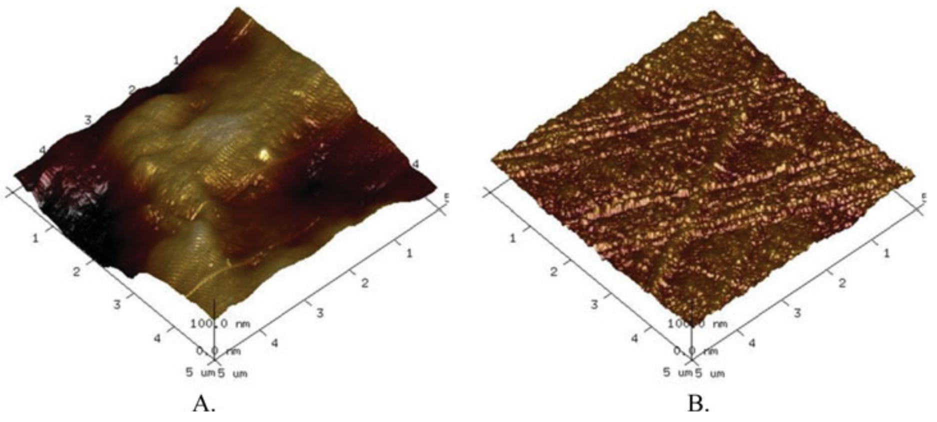

Figure 2. AFM images of the surface of polymer films: A. collagen, B. silk fibroin.

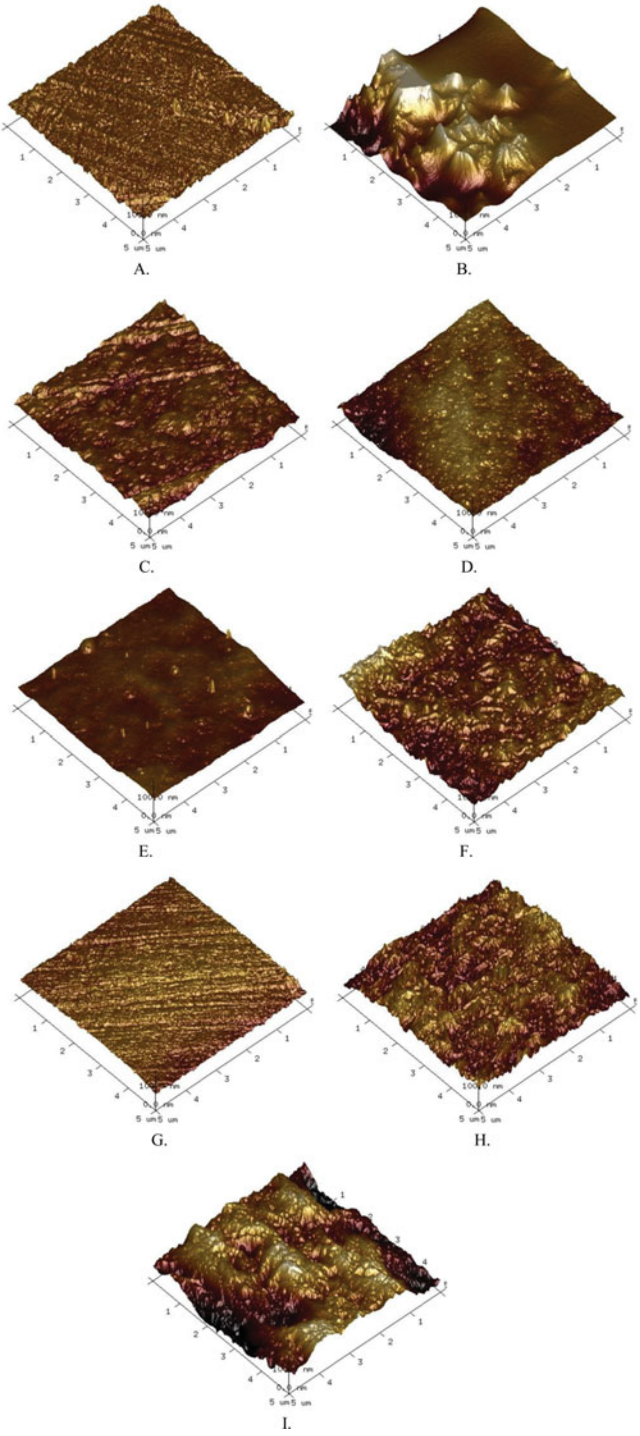

Figure 3. AFM images of the surface of films made of Coll/SF blends in different composition: A. 10/90, B. 20/80, C. 30/70, D. 40/60, E. 50/50, F. 60/40, G. 70/30, H. 80/20, I. 90/10.

Table 3. The roughness parameters (R_q) for films of Col/SF blends of different composition.

$\%_{Col}$	R_q [nm]
0	5.1 ± 0.3
10	5.9 ± 0.3
20	36.7 ± 1.8
30	7.6 ± 0.4
40	7.6 ± 0.4
50	6.7 ± 0.3
60	10.5 ± 0.5
70	7.4 ± 0.4
80	12.4 ± 0.6
90	33.0 ± 1.7
100	26.6 ± 1.3

the AFM image (Figure 3A, C, D) shows the decrease in the number and height of peaks on the film surface. The surface becomes more flat. The observed changes in morphology are related to the interactions between polymeric compounds. Owing to the presence of amine, hydroxyl and carbonyl groups in silk fibroin and collagen, it is possible to combine the chains via hydrogen bonds. The attractive force between components in the blends may lead to a decrease in the size of the microdomains. These conclusions were confirmed by the FTIR and viscometric results as it was shown in our previous paper [25]. Table 3 gives the values of the roughness parameters for the investigated samples. As it can be observed the roughness of the blends increases with the increase of collagen content. The value of the roughness of the Col/SF blend with 10% of collagen is the lowest among the studied films (Table 3). This may indicate an increase in the homogeneity of this blend in comparison to other compositions.

4. Conclusions

All the above results suggest that modification in properties of collagen and silk fibroin in blend is a consequence of the interaction between those biopolymers. Mechanical properties such as tensile strength and Young's modulus were much better for Col/SF blend films than for silk fibroin films. The improvement of mechanical properties of Col/SF blend films can be a result of interactions within a chains and molecular interactions between collagen and silk fibroin.

The results of contact angle and the surface free energy reveal that collagen films are more polar than SF films. In the case of Col/SF blends, the value of the polar component of surface free energy is significantly reduced compared with the pure collagen films, especially in the blends containing the large addition of silk fibroin. This behavior indicates rather hydrophobic character of this surface.

In the case of AFM studies, the surface morphology of films made of pure polymers, and Col/SF is different. Surface roughness increased with the addition of collagen into silk fibroin matrix.

Summarizing, the structure, surface and mechanical properties of collagen and silk fibroin blends depend on the mixture composition.

Acknowledgments

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