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Characterization of silk fibroin 3D composites modified by collagen

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ABSTRACT

Silk fibroin 3D composites with the addition of collagen were prepared through the lyophylisation process. The structure of composites was studied by ATR-FTIR technique and was observed by a scanning electron microscope. Mechanical properties were studied and compared with those of a silk fibroin 3D sponge. Moreover, miscibility studies of silk fibroin with collagen blends of different compositions were investigated using the viscometric method before the lyophylisation process. Viscometric studies indicate that silk fibroin/collagen blends are miscible at any composition at 25 °C. Scanning electron microscopy observations showed that in the lyophilisation process of silk fibroin/collagen blend a porous material can be obtained. The results showed that the addition of collagen to silk fibroin led to the decrease of tensile strength. However, Young modulus increased with an increasing amount of collagen.

1. Introduction

Biomaterials play a very important role in tissue engineering [1]. 3D composites for biomedical application should have good biocompatibility, biodegradability and suitable mechanical properties. Good porosity and pore size are also important, they provide the space for cells to grow, transporting metabolites, nutrients and signal molecules. Porosity gives space and temporary mechanical support for tissue growth [1–3]. For obtaining scaffolds for medical application both natural and synthetic polymers were tested [4,5]. To improve and optimize both chemical and physical properties of biomaterials, polymers used for a scaffold can be mixed together [6–8]. For this purpose also two natural polymers can also be mixed. However, the appropriate solvent is required for the preparation of such blends. Miscible blending of two biopolymers with different physicochemical characteristics may lead to the development of a new biomaterial with unique properties that may present the advantages of each polymer and compensate for the disadvantages of each one [9]. Silk fibroin is a natural polymer produced by various species of silkworm and spiders [2]. *Bombyx mori* cocoons are usually used as a source of silk fibroin. Raw silk contains two proteins, silk fibroin and sericine. However, for the preparation of materials for biomedical application the degummed sericine can be removed [10]. Silk fibroin due to its good mechanical properties and biocompatibility has been used as a biomedical material for a long time [4,11]. The question is, whether silk fibroin can be mixed with another biopolymer, for example with collagen. Collagen is the most abundant protein in mammals. This natural polymer constitutes more than one-third of protein weight in tissue [12]. Collagen can be obtained from different sources such as cow skin or muscles, rat tail tendons, fish scales or skin and even from sea sponges [13]. Materials based on collagen are commonly used in tissue engineering [14,15]. Several materials based on two biopolymers, such as collagen and silk fibroin have already been prepared [16–21]. Composite fibres of collagen and dragline silk protein were also prepared by an electrospinning technique [16]. Some materials were prepared by dissolving collagen in a silk fibroin solution in 60 °C, however, it may cause adverse effects to collagen with a denaturation temperature below 60 °C [3,20,21]. The properties of collagen/silk fibroin scaffolds can be modified using methanol [17,20,21]. It is believed that those materials can be applied in biomedical fields. Although three-dimensional fibroin scaffolds have been prepared using a freeze–drying method previously, they still cannot meet the requirements of tissue engineering and further ideas and study are required. In particular, the results regarding the miscibility of these biopolymers were poorly published. The aim of this work was to study the interactions between silk fibroin and collagen by viscometry technique and to prepare 3D composites based on such blends. Our preliminary study showed that silk fibroin and collagen can be miscible depending on the pH of the solution and ionic strength.

2. Materials and methods

2.1. Preparation of silk fibroin and collagen mixtures

Silk fibroin was obtained from *B. mori* cocoons (Jedwab Polski Sp. Z o.o. company) according to the method described by Kim et al. with slight modifications [22]. Worms were removed from cocoons, then empty cocoons were boiled for 1 h in an aqueous solution of 0.5%

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Na_2CO_3 twice. After the solution was removed, cocoons were boiled in 5% alkaline soap solution for 30 min and then for 20 min in distilled water. This procedure was repeated three times. After removing the sericine, silk fibroin was dried and dissolved in $CaCl_2$:H_2O:C_2H_5OH (molar ratio 1:8:2) at 80 °C for 4 h. The solution of silk fibroin was prepared with a concentration of 4% wt. Collagen was obtained from rat tail tendons. Tendons were washed in distilled water and dissolved in 0.1 M acetic acid for three days in 4 °C [23], the undissolved parts were removed by centrifugation for 10 min at 10,000 rpm. The completely frozen mixtures were lyophilized at −55 °C and 5 Pa for 48 h (ALPHA 1–2 LD plus, CHRIST, Germany). 1% wt solution was prepared by dissolving collagen in 0.1 M acetic acid. Solutions of these two polymers were mixed in weight ratios of silk fibroin to collagen: 90:10, 75:25 and 50:50. Pure silk fibroin was left as a control sample. Each sample was dialyzed in a cellulose tube (SERVAPOR) against distilled water for three days with deionized water changed every day. Finally, the dialysis mixtures were placed in a polystyrene container. The scaffolds were obtained during the lyophylization process for two days. The scaffolds obtained are shown in Fig. 1.

2.2. IR spectroscopy

The interaction between functional groups of silk fibroin and functional groups of collagen and the structure of composites were evaluated by attenuated total reflection infrared spectroscopy using Nicolet iS10 equipped with an ATR device with diamond as a crystal. All spectra were recorded in absorption mode at 4 cm^{-1} intervals and 64 scans.

2.3. Viscometric measurements

Viscometric measurements of dilute polymer solution ($c < 0.5\%$) were carried out in a controlled thermostatic bath at 25 ± 0.1 °C using the Ubbelohde capillary viscometer. The flow times were recorded with an accuracy ±0.01 s. The flow time of each solution was determined as the average of several readings. Before measurements were taken the solutions were filtered through G1 sintered glass filters. The intrinsic viscosity and the interaction parameter values were determined according to the Heller procedure [24,25] from data obtained for solutions at 5 concentrations. The miscibility is estimated by comparison of the experimental and ideal values of b_m and $[\eta]_m$. The values of interaction parameters (b_m) were obtained using the same methods as shown in previous papers [25,26].

2.4. Mechanical properties

Mechanical properties of SF/Col sponges were tested using a Zwick&Roell testing machine. 5 samples of each kind were placed between two discs and pressed. Young Modulus and tensile strength were then measured.

2.5. Scanning electron microscopy

The microstructure of scaffolds was studied using Scanning Electron Microscope (SEM) (LEO Electron Microscopy Ltd., England). Scaffolds were cut with a razor scalpel after being frozen in liquid nitrogen for 3 min.

3. Results and discussion

3.1. IR spectroscopy

Infrared spectra were registered for sponge of silk fibroin and its blends with collagen. ATR-FTIR spectra of specimens studied are shown in Fig. 2. Silk fibroin and collagen are proteins, so both of them give similar spectra in FTIR spectroscopy. Although the miscibility of several polymers in solid state can be confirmed by FTIR spectra, in the blends of two natural polymers with a similar structure this technique is not a very powerful one. In Table 1 the position of typical bands in ATR-FTIR spectra for silk fibroin, collagen and their mixtures are presented. Silk fibroin displays bands at 1645, 1537 and 1241 cm^{-1}, which are characteristic of the amide I, II and III bands of proteins. The amide I absorption arises predominantly from protein amide C═O stretching vibrations, the amide II absorption is made up of amide N–H bending vibrations and C–N stretching vibrations (60% and 40% contribution to the peak respectively); the amide III peak is complex, consisting of components from C–N stretching and N–H in plane bending from amide linkages, as well as absorptions arising from wagging vibrations from CH_2 groups from the glycine backbone and proline side-chains. The amide groups have a characteristic absorption amide bands A and B in the region of 3400–3500 cm^{-1}, however, these bands can be masked by the broad absorption band from the –OH group present in proteins. The amide A band for collagen was observed at 3381 cm^{-1}, whereas for silk fibroin it was observed at 3283 cm^{-1}. For silk fibroin/collagen blends with weight a ratio 50/50 it was observed at 3283 cm^{-1}, the position of this band was similar to the position of amide A observed for silk fibroin. The position of amide I and amide II for silk fibroin and its blends with collagen were also similar. Small differences could only be observed for amide III. It seems that the FTIR technique cannot detect the inter-molecular interactions between silk fibroin and collagen. Usually the inter-molecular interaction between two different polymers through hydrogen bonding can be characterized by FTIR, because the specific interaction affects the local electron density and a corresponding frequency shift can be observed. For silk fibroin/collagen blends, a clear shift of amide bands was not observed. For this reason the viscometry studies of the interaction between silk fibroin and collagen in solution before the liophylisation process were conducted. On the basis of viscometric study one can assess the miscibility of two polymers in the blend in common solvent.

3.2. Viscometric measurements.

All the plots of the reduced viscosity versus polymer concentration (curves not shown) show linear behaviour in the range of concentration studied, indicating that the intrinsic viscosity can be determined by

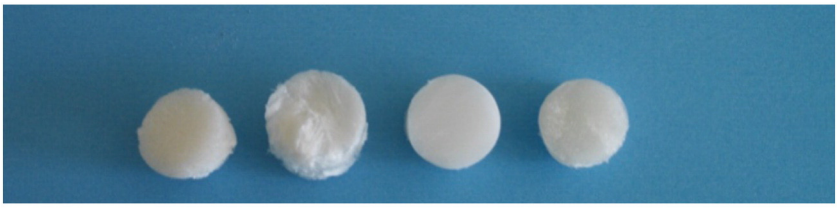

Fig. 1. Scaffolds obtained from: a) silk fibroin, b) SF/Coll (90:10), c) SF/Coll (75:25), d) SF/Coll (50:50).