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Surface and thermal properties of collagen/hyaluronic acid blends containing chitosan

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ABSTRACT

The structure and surface properties of binary and ternary blends containing collagen (Coll), hyaluronic acid (HA) and chitosan (Ch) were investigated by contact angle measurements, thermogravimetric analysis (TGA), scanning electron microscopy (SEM) and atomic force microscopy (AFM). Thin films of Coll/HA and Coll/HA/Ch blends have been formed by casting methods from aqueous acid solutions.

The surface roughness, hydrophobic/hydrophilic character and thermal stability of Coll/HA were changed after addition of chitosan. Thermal stability of binary blends increase upon the addition of chitosan. The results of contact angle and the surface free energy revealed that hyaluronic acid films are more polar than collagen and chitosan films. The surface energy and its polar and dispersive components of binary and ternary blends were calculated and more hydrophilic films were produced by the addition of HA and chitosan, also resulting in more thermally stabile materials. These results demonstrate that collagen interacts with hyaluronic acid and chitosan changing the surface properties of polymer films.

1. Introduction

There has been a growing interest in the use of natural polymers such as proteins and polysaccharides for biomedical and/or cosmetic applications because of their film-forming ability, controlled bioactivity, biocompatibility and biodegradability [1–6]. Hyaluronic acid and chitosan are an interesting hydrophilic natural polysaccharides, showing unique properties such as solubility in an aqueous solution, ability to form complexes and non-toxicity to humans which have resulted in increased interest in investigation and application, e.g., in medicine, pharmacy and cosmetics [7–14]. Collagen is the most abundant natural polymer in animals where it provides the principal structural and mechanical support [6]. Collagen is a very important and attractive protein and its binary or ternary blends can be of significant biomedical and cosmetic application [6,15,16]. A simple approach involves blending with additional inorganic and/or organic compounds, such as biopolymers, synthetic polymers, tannic acid (as a crosslinker), glycerol (as a plasticizer) and montmorillonite [17–26]. This method is usually cheaper and less time-consuming for the creation of materials with new properties than the development of new monomers and/or new polymerization routes. An additional advantage of polymer blends and composites is that the materials can be tailored by combining component polymers and changing the blend composition. Thus, the main purpose of our studies was to prepare the binary and ternary systems with better physico-chemical properties that can be used in the material's potential applications in medicine, pharmacy or cosmetics. The miscibility of collagen and hyaluronic acid with and without chitosan and chitosan and hyaluronic acid with and without collagen has been studied previously by us by viscometric method, tensile tests and ATR-FTIR technique [27,28]. It was found that collagen and hyaluronic acid are miscible due to the intermolecular interactions between components. In the case of chitosan and hyaluronic acid blends and ternary blends, the polymeric components were partially miscible.

Studies on the physico-chemical and biological properties of collagen and hyaluronic acid with and without chitosan in the solid state have been reported [29–34]. For collagen/hyaluronan/chitosan composite sponges [32], the results showed that the 9:1:1 mixing ratio of collagen, hyaluronan and chitosan was the optimal ratio for the manufacture of complex scaffolds in different fields of tissue engineering based on its properties, cell biocompatibility and low cost. Cai et al. [31] have described the coagulation property of hyaluronic acid-collagen/chitosan complex film. The obtained results indicated that the HA-Col-I/Ch film possessed promising coagulation property, cell compatibility and anti-bacterial property, and the potential in future clinical application such as wound healing and bandage.

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The aim of our studies was to prepare and investigate the surface properties and structure of natural polymers – collagen, hyaluronic acid, chitosan and their binary and ternary blends by means of contact angle measurements, scanning electron microscopy, atomic force microscopy and thermogravimetric analysis.

2. Materials and methods

Hyaluronic acid (HA) and chitosan (Ch) are commercial polymers and were obtained from the Aldrich company and had a viscosity average molecular weight of 1.8×10^6 and 0.59×10^6, respectively. The degree of deacetylation was 78% for Ch. Collagen (Coll) was extracted in our laboratory from tail tendons of young rats. Tendons were washed and dissolved in 0.1 M acetic acid. The obtained solution was then spun at 10000 rpm in a Sorvall centrifuge and the soluble fractions were decanted and lyophilized [20]. Collagen, hyaluronic acid and chitosan were separately solubilized in aqueous acid solutions (0.1 mol dm^{-3} HCl for HA and 0.1 mol dm^{-3} CH_3COOH for Coll and Ch). Coll/HA blends were prepared from mixed polymer solutions. The composition of Coll/HA was 80/20, 50/50 and 20/80. The chitosan solution was added in the different ratios (10–90%) based on the Coll/HA blend in 80/20 wt ratio.

The blend composition in this study was chosen on the base of results obtained in our previous study [28]. Our previous study [28] showed that for weight ratio of Coll/HA binary blend higher than 0.5 or equal to 0.5, the miscibility of polymeric components is better than for those of other weight ratios. Therefore, the weight ratio of Coll/HA was kept to 80/20, and then the binary blend was blended with different amounts of chitosan solution in this study. These solutions were cast to prepare the polymer films.

The contact angles (Θ) for diiodomethane (D) and glycerol (G) on the surfaces of polymer films were performed at room temperature using the DSA10 goniometer of Krüss GmbH (Germany), equipped with software for the drop shape analysis. The droplets of the probe liquid (high purity, volume of 3 mL) were deposited using a micro-syringe; the drop image was recorded with a video camera and then digitalized. Using the instrument's software, the drop shape was fitted to obtain the contact angle between the solvent and the surface. Each contact angle represents an average of 10 measurements. The surface free energy was calculated using the Owen–Wendt method [35].

Thermogravimetric analysis (TGA) was carried out using a Thermal Analysis SDT 2960 Simultaneous TGA-DTA analyzer from TA Instruments in the temperature range of 20 °C to 650 °C at a heating rate of 20 °C/min in nitrogen. From the thermogravimetric curves, we obtained the characteristic temperatures of decomposition: temperature of initial decomposition (T_{di}) and temperature at maximum decomposition rate (T_{max}).

AFM imaging in the tapping mode and ambient conditions was done using a multimode scanning probe microscope with a Nanoscope IIIa controller (Digital Instruments Santa Barbara, CA). Surface images, using scan widths ranging from 1 mm to 5 mm, with a scan rate of 1.97 Hz were acquired at fixed resolution (512×512 data points). The roughness parameter was calculated for scanned area (5 mm × 5mm) using Nanoscope software. The AFM images and roughness calculations were obtained for different sample places and the most typical areas are presented.

The morphology of the samples was studied using Scanning Electron Microscopy (SEM) LEO Electron Microscopy Ltd. England.

3. Results and discussion

Wetting experiments were carried out using diiodomethane (D) and glycerol (G) as test liquids to get information on hydrophobic/hydrophilic character of polymer films. When a contact angle is between 0° and 90°, it results in spreading of the drop due to the molecular attraction. The value of contact angle greater than 90° indicates the liquid tends to bead or shrink away from the surface. Tables 1 and 2 give the corresponding values of contact angles, surface free energies and their polar and dispersive components for collagen, hyaluronic acid, chitosan and their blends. As it can be observed, the surface of pure hyaluronic acid is rather hydrophilic, as the glycerol contact angle was lower (~47°) than that for diiodomethane (~60°). The highest contact angle for glycerol (~76°) was found for the collagen film, which suggests that this surface is the most hydrophobic. For Coll/HA binary blends, the glycerol contact angles of blend films increase from 47.3° to 70.6° and 72.3° after the addition of collagen. These results indicate that the wettability of binary blend films decreased.

Owens – Wendt method [35] was used to calculate the surface free energy. The hyaluronic acid film has the highest polarity among the selected polymers because it has the highest polar component of its surface free energy (Tables 1 and 2). The lowest polarity is characteristic for the collagen film and the Coll/HA film blend, which suggests that there are fewer interactions between the reactive groups of polymers. Thus the polar groups of polymers have been hidden below the surface of film. After the addition of chitosan into the binary blend we observed the decrease of contact angle for glycerol (~47–64°, Table 2). The lowest value of glycerol contact angle is found for the ternary blends with the weight fraction of chitosan between 0.7 and 0.9 which suggests that the surface is the most hydrophilic among the investigated blend films. The surface free energies have much higher values for the Coll/HA/Ch ternary blends than for the pure collagen and the Coll/HA blend.

The computation of dispersive and polar components of surface free energy gave us more detailed information on surface properties of investigated blend films (Table 2). As it can be seen, the dispersive component decreases and simultaneously the polar component of surface free energy increases with the increasing content of chitosan in the ternary blends. Generally, for all the ternary blends the values of polar component are bigger than for pure collagen and binary blends but it is smaller than for pure hyaluronic acid film. The addition of chitosan into Coll/HA makes the surface of this blend more hydrophilic. Thus, probably part hydrophilic groups of chitosan, such as hydroxyl and amino groups, come out on the surface of film. The results suggested that the hydrophilicity of collagen blend films was much improved by the addition of hyaluronic acid and chitosan. However, the observed changes in the polarity are quite irregular with multiple maxima and minima. This behavior is related to the interactions between polymeric components. In our previous study, we found that for the ternary blends, the components were poorly miscible [28]. This may be due to the stronger electrostatic interactions and/or repulsive force between the components in the blend. These reasons could be responsible for the irregular value of the dispersive and polar components of surface free energy.

Changes in the thermal stability of pure polymers and their blend films under nitrogen flows were examined by thermogravimetric analysis (TGA). The weight loss curves of the films as a function of temperature are shown in Figs. 1 and 2 and Table 3. The TGA curves presented for the HA sample and Coll/HA blends consist of three stages, while the collagen curve shows two stages (Fig. 1 and Table 3). The first stage at 30–130 °C was due to the loss of moisture and residual acetic acid and it showed an approximate 11%, 16% and 10% loss in weight for HA, Coll and Coll/HA blends, respectively. These results indicate that the binary Coll/HA blends entrap a lower amount of water in their structure in comparison to the pure collagen. The main decomposition of pure hyaluronic acid was in the range between 200 °C and 300 °C with a center at 214 °C, which was attributed to the partial breakage of the molecular