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Modification of 3D materials based on chitosan and collagen blends by sodium alginate

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ABSTRACT

The aim of this work was to study the properties of chitosan/collagen blend modified by sodium alginate. 3D composites of chitosan/collagen blend were prepared by freeze drying technique.

The results showed that sodium alginate modified the properties of the 3D composite of chitosan/collagen blends. The porosity decreased and the mechanical properties were improved. Mechanical parameters increased with an increasing amount of sodium alginate in chitosan/collagen composite. Materials after cross-linking by sodium alginate can be used in medical applications to produce implants or wound dressings.

KEYWORDS

biomaterials; collagen; chitosan; sodium alginate; mechanical properties

1. Introduction

Collagen and chitosan are natural polymers which are widely used in tissue engineering due to their high biocompatibility and other interesting properties [1–4]. Collagen and chitosan can be used together as a blend to fabricate biomaterials [5]. Nevertheless, due to its weak mechanical properties, other natural polymers or cross-linking agents need to be added to improve the properties of the collagen composite for tissue engineering [6]. Tissue engineering refers to the growth of new tissue using living cells based on the structure called a scaffold [7]. Biopolymer based materials are not solely used for scaffold preparation, but also as wound dressings and for controlled drug delivery [8].

Biopolymers for tissue engineering application must have appropriate mechanical properties for the target [9]. Sometimes it is essential to use a cross-linking agent to improve the properties of the material [8]. Cross-linking agents are compounds which interact with the natural polymers and change the material structure. Sodium alginate is an anionic polysaccharide which can interact with cationic natural polymers e.g. chitosan due to electrostatic interaction [10–13]. It has been found that composites of sodium alginate and chitosan had both, low toxicity and were bioresorbable [10–14].

Collagen is an amphiphilic polymer with cationic and anionic groups that are present in the structure. As a result it can interact with chitosan as well as sodium alginate by the formation of strong ionic interactions [15,16]. Various research has already been done to modify collagen [9,15] and chitosan [17,18] with sodium alginate. The new aspect of our experiment is the addition of sodium alginate to the mixture of chitosan and collagen and the preparation of 3D

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composites based on such blends. It is worthy of study whether the addition of sodium alginate to chitosan/collagen blend can offer any added value to the preparation of 3D composites based on such blend. All of the natural polymers used in experiment are biocompatible and the use of their mixture does not cause the enhance of material toxicity.

2. Material and methods

2.1. Sample preparation

Collagen was obtained from rat tail tendons in the laboratory. Chitosan ($M_v = 5.4 \times 10^5$ g/mol; DD = 77%) was purchased from Fluka. Collagen and chitosan solutions with appropriate concentrations were prepared [3]. Chitosan and collagen mixtures were prepared by mixing two solutions in the weight ratio 50/50. Sodium alginate (SA) as 1% solution in 0.1M acetic acid was added as a cross-linking agent in the weight ratios 2 and 5%. Total miscibility was achieved by adding a few drops addition of 2M hydrochloric acid. The addition of hydrochloric acid did not change the final pH of the mixture. Solutions were mixed and put into the polystyrene container and then placed in a freezer at −80°C. Frozen mixtures were lyophilized at −55°C and 5Pa for 48h (ALPHA 1–2LD plus, CHRIST, Germany). Chitosan/collagen composites as well as a pure chitosan and collagen scaffolds were left as control probes.

2.2. Infrared spectroscopy

Spectra of attenuated total reflected infrared spectroscopy were obtained for 3D structures. FTIR spectra were obtained using a Genesis II FTIR spectrophotometer (Mattson, USA) equipped in ATR device with zinc selenide crystal [4]. All spectra were recorded in absorption mode at 4 cm^{-1} interval and 64 scans.

2.3. Density and porosity

The density and porosity of composites were measured by isopropanol displacement, because it does not wet the sample [4]. Samples were put into the known volume of isopropanol. After 5 minutes the change in volume of isopropanol-impregnated scaffold was measured. The sample was removed from the solution and again the difference in isopropanol volume was determined. The density of the porous sample (d) was calculated:

$$d = \frac{w}{v_2 - v_3} \tag{1}$$

where W is sample weight, V_2 is the total volume of isopropanol and isopropanol-impregnated scaffold, V_3 is the isopropanol volume after sample removing. The porosity of the scaffold was calculated using the equation:

$$\varepsilon = \frac{v_1 - v_s}{v_2 - v_3} * 100\% \tag{2}$$

where V_2, V_3 as above, V_1 is the initial volume of isopropanol.

2.4. Mechanical properties

Mechanical properties were measured by mechanical testing machine (Z.05, Zwick/Roell, Germany) for each kind of sample. Cylindrical samples with a diameter of 15 mm and height of 14 mm were prepared for mechanical testing. All measurements were carried out in room temperature and humidity. The cross-head speed was set at 2 mm/min. The compressive modulus is a Young modulus for the compression process, where it determinates the stiffness of an elastic composite. It was calculated from the slope of the stress-strain curves in the linear region (strain from 2 to 5%). For each kind of composite, at least five samples were tested.

2.5. Swelling of specimens

Swelling behavior was measured by immersing the composites in phosphate-buffer saline (PBS pH = 7.4) solution. After 2; 4; 8; 24; 48 and 72 h samples were gently dried by putting them between two sheets of paper and weighted. Weight changes were then calculated using the equation:

$$swelling = \frac{(m_t - m_0)}{m_0} * 100\,[\%] \tag{3}$$

where m_t is the weight of the blend after immersion in PBS and m_0 is the weight of a blend before immersion. Separate samples were used for each incubation time.

2.6. SEM microscopy

The morphology of the samples 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.

2.7. Moisture content

The moisture contents were determined by drying samples in the oven at 105°C until they reached a constant weight. Results were expressed as grams of water in 100 g of the dry sample.

3. Results and Discussion

3.1. Infrared spectroscopy

Characteristic peaks of Amide A, Amide I, Amide II bands and also peak of C–O–C (1074 cm^{-1}) are found in infrared spectra of chitosan/collagen blends (Table 1). After the addition of sodium alginate peaks are shifted because new interactions between polymers are formed. Hydrogen bonds are present between amine and carboxylic as well as hydroxyl groups. The functional parts of polymers as amine and carboxylic groups can be ionized and then ionic interactions are formed and the scaffold structure is stabilized. The intensity of peaks changed after addition of sodium alginate, where the peak at 1122 cm^{-1} decreased as a result of ionic interactions between polymers. Moreover, after the addition of sodium alginate one more peak at 1405 cm^{-1} can be observed from the C=O carboxyl anions, which is characteristic for sodium alginate [19–21].

Table 1. Characteristic groups in FTIR spectra of chitosan/collagen composite (CTS/Coll) with 5% addition of sodium alginate (SA).

Wavenumber [cm^{-1}]	Functional group
CTS/Coll	
3338	Amide A
1650	Amide I
1550	Amide II
1074	C–O–C
CTS/Coll/SA	
3334	Amide A
1652	Amide I
1552	Amide II
1405	C=O
1074	C–O–C

After the addition of sodium alginate to chitosan and collagen composites characteristic peaks of carboxyl anions can be observed in the infrared spectra of all studied composites. It showed that the structure of chitosan/collagen blend was modified by adding sodium alginate as a cross-linking agent. FTIR spectra confirms the presence of sodium alginate in the composite and suggest the interaction between components.

3.2. Density and porosity

Density and porosity for materials based on the blends of chitosan and collagen with sodium alginate are shown in Table 2. Density and porosity are parameters, which have to be determined for porous composites. These parameters are inversely proportional. 3D sponges obtained by mixing chitosan and collagen has higher density than those that are obtained from pure polymers. The 2% addition of sodium alginate is not sufficient to improve the density of the material, however, after 5% addition an increase of density can be observed. The highest porosity can be observed for the collagen sample, which has at the same time the lowest density.

3.3. Mechanical properties

Compressive modulus and the compressive strength for chitosan/collagen composites with sodium alginate and without it are shown in Table 3. An addition of 2% and 5% of SA was used and the properties of the materials were compared.

The addition of sodium alginate modifies the mechanical properties of a composite, such as compressive modulus and compressive strength. The lowest mechanical properties were found for pure collagen 3D material. For the mixture of chitosan and collagen, mechanical properties were lower than for pure chitosan. The mechanical properties of chitosan/collagen

Table 2. Density (d) and porosity (ε) of chitosan (CTS) and collagen (Coll) composites with 2% (CTS/Coll/2SA) and 5% (CTS/Coll/5SA) sodium alginate addition.

Specimen	d [mg/cm^3]	ε [%]
CTS	59.01	86.50
Coll	20.10	91.40
CTS/Coll	74.77	76.00
CTS/Coll/2SA	71.31	56.79
CTS/Coll/5SA	81.34	20.99

Table 3. Compressive modulus (E_{mod}) and compressive strength (F_{max}) for chitosan/collagen (CTS/Coll) composites in 3D structure with 2% (CTS/Coll/2SA) and 5% (CTS/Coll/5SA) addition of sodium alginate.

Specimen	E_{mod} [kPa]	F_{max} [kPa]
CTS	0.73 ± 0.17	12.12 ± 0.47
Coll	0.24 ± 0.07	1.71 ± 0.19
CTS/Coll	0.31 ± 0.15	5.96 ± 0.26
CTS/Coll/2SA	0.37 ± 0.09	7.57 ± 0.43
CTS/Coll/5SA	0.44 ± 0.11	8.24 ± 0.09

blends were improved in comparison with the materials based on collagen. Compressive modulus as well as the compressive strength increase with an increasing amount of sodium alginate added to chitosan/collagen blend.

3.4. Swelling of specimens

The swelling behavior of the polymer depends on both the polymer structure and liquid nature as well as on the degree of cross-linking of polymer. Swelling behavior is also a parameter, which determine the polymer composite properties, especially for hydrophilic polymers. The conditions of solution as pH or its hydrophilic or hydrophobic character have influence on the stability of 3D composites. Phosphate-buffer saline (PBS) solution has pH = 7.4, which is relevant to the pH of blood. The use of such solution allows to examine the behavior of material after its application inside the body. Both, chitosan and collagen are hydrophilic biopolymers which can swell in aqueous media. Swelling behavior for composites of chitosan/collagen with and without sodium alginate is shown in Table 4. The highest swelling was after 2 h of immersion for each kind of sample. Swelling decreases with an increasing immersion time. The highest swelling behavior can be observed for the chitosan sample and for collagen the swelling is the lowest. An increasing amount of sodium alginate leads to a swelling increase. Swelling behavior is very important when biopolymer materials are going to be applied as a matrix in drug delivery.

3.5. SEM microscopy

The structure of composites based on the blends of chitosan and collagen before and after the addition of sodium alginate can be observed by SEM. SEM images of chitosan and collagen composite in ratio 50/50 with the addition of sodium alginate are shown in Figure 1. In each case, a porous structure was obtained in which pores were interconnected. There is no significant difference in the size of the pores observed in SEM images. However, after cross-linking with sodium alginate the porosity of the material decreased, as it was shown in porosity

Table 4. Swelling behavior of chitosan and collagen (CTS/Coll) composites in 3D structures with 2% (CTS/Coll/2SA) and 5% (CTS/Coll/5SA) of sodium alginate.

	Swelling [%] after time of immersion in PBS [h]					
Specimen	2	4	8	24	48	72
CTS	2808			sample dissolved		
Coll	232	190	162	204	205	185
CTS/Coll	508	368	570	382	598	415
CTS/Coll/2SA	1174	254	531	161	323	723
CTS/Coll/5SA	1278	830	770	275	362	606

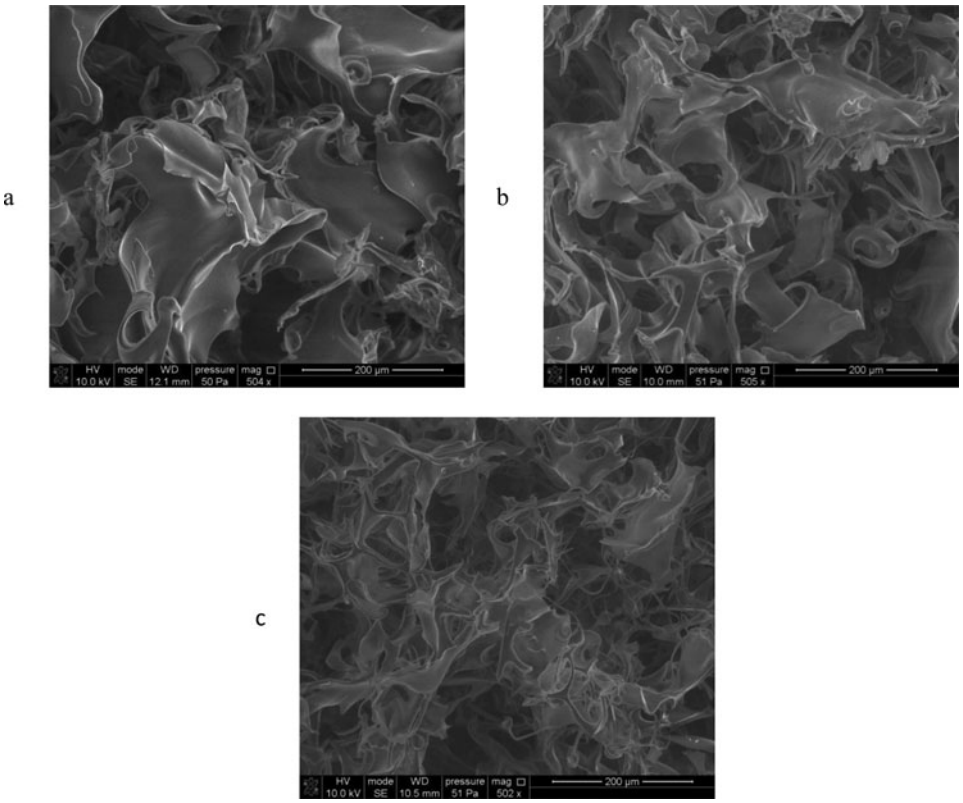

Figure 1. SEM images of a) chitosan and collagen in ratio 50/50 b) chitosan with collagen in ratio 50/50 with 2% addition of sodium alginate c) chitosan with collagen in ratio 50/50 with 5% addition of sodium alginate.

measurement by isopropanol displacement (see part 3.2). It suggests that SA modified the structure of composite even if SEM microscopy cannot show the differences in pore size. It seems that only on the surface of the composite the pore size is similar, but inside the composites there are pores with different size.

3.6. Moisture content measurements

A moisture content measurement was made for each kind of sample, with and without the addition of sodium alginate. The water content was calculated for 100 g of sample. The results are shown in Table 5.

The moisture content measurement is important if one wants to consider the water presence in the sample. It can be important for several biomedical application when materials will

Table 5. Water content (g/100g) in chitosan, collagen and their composite with addition of 2 and 5% of sodium alginate.

Specimen	g/100g
CTS	29.00
Coll	19.51
CTS/Coll	32.80
CTS/Coll/2SA	31.22
CTS/Coll/5SA	29.48

be applied in wet condition. The water molecules were absorbed by composites and during the measurement the water content could be determined. The composite based on the blends of chitosan and collagen mixture has higher water content than 3D sponge made of pure collagen and pure chitosan. The addition of the sodium alginate causes a decrease in moisture content in the composite. The lowest moisture content in the condition of the experiment was observed for the collagen sample.

Conclusion

The porous 3D scaffolds made of the blends of chitosan and collagen can be obtained by lyophilization process. The properties of chitosan/collagen blends such as mechanical parameters, swelling, density and porosity can be modified by sodium alginate. Sodium alginate can interact with chitosan and collagen due to the electrostatic interactions and hydrogen bonds. Materials after cross-linking by sodium alginate can be used in medical applications to produce implants or wound dressings, where the biological properties, such as biocompatibility and biodegradability, can meet the requirements appropriate for the applications.

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