Characterization of konjac glucomannan-gelatin IPN physical hydrogel scaffold

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Abstract. A novel IPN hydrogel scaffold is prepared by freeze-drying method, in which konjac galactomannan (KGM) and gelatin are physically crosslinked respectively. This scaffold is thermostable, and the structure of this scaffold is analysed by scanning electron microscope, Fourier transform infrared spectrum, and X-ray diffraction method. The FT-IR results show that hydrogen bonds are formed between KGM and gelatin molecules, which hinder the formation of their respective crosslinking. This is consistent with the XRD results that the crystallinity gets lower in the IPN gels compared with pure gelatin and KGM gels. The morphologies of freeze-dried hydrogels are observed by SEM and the mechanical properties of the scaffolds are tested to analyse the relationship between the structures and properties. Although this novel IPN hydrogel is physical gel, it shows rubber-like performance as chemical gels. And it is nontoxic, so it can be used as the scaffold for cartilage tissue engineering that embedded in human bodies.

1. Introduction

Gelatin is a kind of polypeptide derived from collagen hydrolysis, and is widely used in food, drug delivery, and tissue engineering [1]. When temperature decreases below 35 °C, coil-helix transition of gelatin molecules will happen in solution, which causes the sol-gel transition [2]. The structure of formed triple helices is identical with that of collagen [3]. Triple helices are formed by self-assembly of gelatin molecules due to the action of hydrogen bonds [4]. When temperature increases above 35 °C, triple helices will dissolve in water, and the gel transforms into the sol. The body temperature of human is above 35 °C. Hence, the physical gel of gelatin is unstable as a kind of scaffold embedded in human bodies. Therefore, chemical crosslinks are involved in gelatin gels to resist melting at body temperature. The most common crosslinking agent is glutaraldehyde [5]. But glutaraldehyde is toxic [6]. Hence, genipin becomes an alternative recently, which has low toxicity [7].

Many polymers are compounded with gelatin to form new hydrogels for the purpose of improving the hydrogel properties. The additive includes natural polymers and synthetic polymers. Chitosan, hyaluronic acid, alginate and agar are the commonly used natural polymers [8-11]. Synthetic polymers such as polyvinyl alcohol and poly (ethylene glycol) are also used commonly [12-14]. Konjac galactomannan (KGM) is a kind of polysaccharide derived from amorphophallus konjac plants [15]. When heated in alkaline solution, deacetylation will happen on KGM, and the KGM molecules are crosslinked simultaneously [16, 17]. These cross linkages are thermal stable, will not damage even
heated [18]. KGM is non-toxic, and with good biocompatibility, so KGM hydrogels are used in food, pharmaceutical and biological tissue engineering [18].

In this study, gelatin and KGM are crosslinked respectively to form interpenetrating polymer network (IPN). The schematic is shown in Fig. 1. The physical properties and morphology of the hydrogels are studied by SEM, XRD, FT-IR and mechanical testing machine. The results show that hydrogen bondings are formed between KGM and gelatin, which hinder the formation of their own crosslinking. The structural properties also affect the mechanical properties of the hydrogels.

![Figure 1. Schematic of prepared KGM-gelatin IPN hydrogel](image)

2. Experimental

2.1 Materials
All the chemical reagents used were obtained from commercial sources in China. The gelatin was purchased from Sino Pharm Chemical Reagent Co., Ltd, Shanghai, China. The KGM was purchased from Shiyan Huaxianzi Konjac Productions Co., LTD, Hubei, and China. The content of galactomannan is more than 95%.

2.2 Preparation of hydrogels
Three kinds of hydrogels were designed and prepared for the contrast. The ingredients of the hydrogels are: 2.0 g KGM; 1.0 g KGM and 1.0 g gelatin; 2.0 g gelatin. A certain amount of gelatin was soaked in 50 mL deionized water for at least 2 h, for making gelatin swell sufficiently. Then the water was heated to 45 °C and stirred simultaneously to make gelatin dissolved completely. After that, 0.1 mL ammonium hydroxide with the concentration of 25% was added to the solution, and then, the mixed solution was stirred evenly. KGM was added to the solution subsequently with severely stirring until the solution became homogeneous. Then the temperatures of the solutions were raised to 70 °C and kept for 24 h. After that, the samples were transferred to the fridge with the temperature of 4 °C and kept for 24 h, and then the hydrogels were completely prepared. The dry samples were prepared by freeze-drying method at the temperature of -40 °C.

2.3 Characterization

2.3.1 Morphology observations
The surface morphologies and internal structures of freeze-dried hydrogels were observed by a scanning electron microscope (SEM Quanta 200, FEI, USA).

The samples were freeze-dried at -40 °C to maintain the porous structure without any collapse.

The apertures of pores were measured by geometrical observation on screen.

2.3.2 FT-IR analysis
Fourier transform infrared spectrum experiments were performed on Bruker TENSOR27 infrared spectrometer (Germany), 4000~400 cm-1, Sweep frequency of 10 times, and Spectral resolution of 4 cm-1.
2.3.3 X-ray diffraction studies
Wide-angle X-ray diffraction patterns were recorded on an X-ray diffractometer (D/MAX-3B, Rigaku Denki, Japan) in the range of $2\theta=5$–$90^\circ$ with Cu $k\alpha$ radiation ($\lambda=1.5406\times10^{-10}$ m) at 40 kV and 30 mA.

2.3.4 Mechanical tests
Scaffolds were made to 10 mm $\times$ 10 mm $\times$ 10 mm cubes for mechanical tests. The tests were performed on HY-0230 computer controlled electronic universal testing machine (Shanghai HengYi Precision Instrument Co., Ltd., China).

3. Results and discussion

3.1 FT-IR analysis
In order to confirm the reactions among the functional groups, FT-IR spectra of gelatin, KGM, and composite of gelatin and KGM freeze-dried gels are shown in Fig. 2. The characteristic absorption bands of mannose in the KGM (curve a) appear at 876 and 810 cm$^{-1}$, and the peak at 895 cm$^{-1}$ in the KGM corresponds to $\beta$-glucosidic bonds. These absorption peaks mentioned above also appear in the composite of gelatin and KGM (curve b). Hence, it can be concluded that the primary structures of KGM keep intact in the composite. Moreover, it can be seen from curves a and b that the peaks at around 2854 and 1740 cm$^{-1}$ which represent acetyl groups do not appear. This is because the ammonia solution is weakly alkaline, which made deacetylation happened on KGM. The FT-IR spectrum of gelatin (curve c) showed strong absorption bands at 3330 cm$^{-1}$ (N-H stretching), 1655 cm$^{-1}$ (amide I, C=O stretching), 1547 cm$^{-1}$ (amide II, N-H bending), and 1240 cm$^{-1}$ (amide III, C-N stretching). Theses mentioned absorption bands can also be found in curve b. Therefore, it can be concluded that the primary structures of gelatin keep intact in the composite gel.

![FT-IR spectra of KGM (a), composite of gelatin and KGM (b), and gelatin (c) freeze-dried gels](image_url)

Figure 2. FT-IR spectra of KGM (a), composite of gelatin and KGM (b), and gelatin (c) freeze-dried gels

It can be seen from Fig. 2 that the location and intensity of some bands on curve a, b and c are different. The absorption band at 3427 cm$^{-1}$ on curve a corresponds to O-H stretching vibration, but it appears at 3413 cm$^{-1}$ on curve b. It shows significant red shift of O-H band in the composite gel compared with the KGM gel. The reason is analyzed as follows. KGM has a large number of hydroxyl groups, which can easily form hydrogen bonding with KGM molecules and gelatin molecules. There are a huge number of peptide bonds in gelatin molecules, and the six atoms which form the peptide bond are in one plane. Therefore, C-N bond has the characteristics of double bond, and cannot rotate freely. The triple helices formed in gelatin gels are caused by hydrogen bonds, which occur among carbonyl groups (C=O) on the amino acid residues and amino groups (N-H) on the nearby amino acid residues. The hydrogen bonds have the properties of saturation and directivity, and the bond angels are around 180$^\circ$ in most cases. Hence, it can be concluded that the hydrogen bonds formed between KGM and gelatin molecules attribute to the action between the hydroxyl groups of KGM and carbonyl groups of gelatin. It can be seen from Fig. 2 that the absorption band of amide I on curve b shifts to
1645 cm$^{-1}$ compared with 1655 cm$^{-1}$ on curve a, which also indicates that the carbonyl groups of gelatin interact with the hydroxyl groups of KGM.

3.2 X-ray diffraction studies
XRD patterns of KGM (curve a), composite of gelatin and KGM (curve b), and gelatin (curve c) gels are shown in Fig. 3. It can be seen in curve a that the crystalline peaks and dispersion peaks of amorphous phase stack up together. It has been found that the addition of ammonia to KGM solutions is required in order to remove the acetyl groups (deacetylate KGM) and induce the formation of a hydrated gel-forming presudo-fibrillar precipitate [17]. The gelation of the KGM molecules possibly occurred through the formation of a network structure supported by hydrogen bonds. Hence, it was considered the acetyl groups associated with KGM inhibited gel formation [17]. The presence of mild alkali in a KGM solution cleaved the acetyl groups and resulted in heat-stable elastic gels, which retained their structure under various conditions. The electron diffraction diagram of KGM gel illustrated the mannan II-type crystallization [17].

![Figure 3. X-ray diffraction patterns of KGM (a), gelatin-KGM (b), and gelatin (c) freeze-dried gels](image)

3.3 Mechanical tests
The elastic moduli were measured when the compressive deformation was 10%, which were respectively 13.336 kPa of KGM gels, 2.813 kPa of composite gels and 21.648 kPa of gelatin gels. Even the gelatin gels exhibit the highest elastic modulus among the three kinds of hydrogels, the thermal-reversible characteristic limited their applications. The gelatin gels fractured when the compressive strain reached 38%, and the corresponding compressive strength was 11.03 kPa. The KGM and the composite hydrogels did not fracture even the compressive strain reached 70%, and the elastic modulus increased with the increase of compressive strain.

It was found that the elastic modulus reduced a great value of the composite hydrogels compared with KGM gels. The reason is analyzed as follows. Generally, the moduli of polymer gels depend on the crosslinking density and crosslinking types. KGM and gelatin gels are both physical gels, and the crosslinking zones are formed by hydrogen bonds. The entanglement of KGM and gelatin molecular chains and the association between gelatin and KGM molecules by hydrogen bonds hindered the formation of respective crystallization of KGM and gelatin. Therefore, the crosslinking density decreased and the crosslinking zones shortened, which decreased the moduli of the composite hydrogel.

3.4 Morphology observations
The SEM images were obtained to characterize the microstructure of the freeze-dried hydrogels and are presented in Fig. 4.
This suggests that the hydrogel matrices are porous, with a three-dimensional interconnected microstructure by virtue of the freeze-drying step with the pores being the result of ice crystal formation. The apertures in the gelatin matrices are around 200–300 μm wide estimated from the micrographs. In contrast, the apertures in KGM and composite gels are not uniform as that in gelatin gels, and spread from around 300 μm to 500 μm. It is seen that gelatin gels have the strongest walls which make the shapes of the pores uniform. This is due to the materials characteristics. As discussed in section 3.3, gelatin gels have better stiffness and worse toughness compared with KGM gels. The stiffness of the structure can maintain its shape during the freeze-drying process. While the KGM gels are the opposite. They have good elasticity and toughness, the walls of the pores are not stiff as that of gelatin gels, which causes the irregular pores in the freeze-drying process. The composite gels also have irregular pores, but are better than that of KGM gels. It is considered that the gelatin networks in the composite gel play a supporting role.

4. Conclusions
Novel IPN hydrogels were prepared by physically crosslinking gelatin and KGM respectively. Gelatin physical hydrogel will melt to sol at body temperature, but the IPN hydrogel can maintain its structure under various conditions, although it is still physical gel. This should be attributed to the KGM networks formed in the IPN hydrogels.

Hydrogen bond was formed between the carbonyl group of gelatin and hydroxyl group of KGM, which is analyzed by FT-IR method. The entanglement of molecular chains and the association between gelatin and KGM molecules by hydrogen bonds hindered the formation of their respective crystallization, which can be proved by XRD patterns. And this directly caused the crosslinking density decreased and the crosslinking zones shortened in the IPN hydrogel. So that the compressive modulus of IPN hydrogel was lower than that of KGM hydrogel. Nonetheless, the toughness of the IPN hydrogel was much higher than that of gelatin hydrogel.

This novel IPN hydrogel is a kind of physical gel, but it shows rubber-like performance as chemical gels. And it is nontoxic, so it can be used as the scaffold for cartilage tissue engineering that embedded in human bodies.

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