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Condensed state structure and biocompatibility of the konjac glucomannan/chitosan blend films

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Abstract

Specialised blend films have been prepared by blending 1% w/v konjac glucomannan aqueous with 1% w/v chitosan solution in acetate solution and drying at room temperature for 24 h. The condensed state structure and miscibility of the blend films were studied by Fourier transform infrared spectroscopy, scanning electron microscopy, differential scanning calorimetry, and wide-angle X-ray diffraction. The results indicated that the blend film obtained from an 80/20 mixing ratio of konjac glucomannan and chitosan derivate showed the highest miscibility and blend homogeneity, and that strong intermolecular hydrogen bonds took place between the amino groups of chitosan and the hydroxyl groups of konjac glucomannan; thus the tensile strength also achieved its maximum in this ratio. The cell morphologies on the pure and blend films were examined by light microscopy and cell viability was studied by using MTT assay. The results showed that the particular blend film was more suitable for the cell culture than the pure konjac glucomannan film, and that the cells cultured on this blend film had greater spreading coefficients than that of the pure konjac glucomannan film. As a result of the good mechanical properties, miscibility and biocompatibility, the blend film is a promising biomaterial matrix.

Keywords: Konjac glucomannan; Chitosan; Miscibility; Biocompatibility; Blend film

1. Introduction

A wide variety of biomaterials with different physical-mechanical, chemical and biochemical properties depending on the biomedical application have been developed in the last 20 years. Directly related to the chemical and biochemical characteristics of these biomaterials was their biocompatibility, and it was defined as 'the quality of not having toxic or injurious effects on biological systems' (Serrano et al., 2004). Recently, biocompatibility had been considered as 'the ability of a material to perform with an appropriate host response in a specific application' (Serrano et al., 2004), taking into account the interactivity between the biomaterial and the host. Among the prominent applications for biomaterials are: controlled drug delivery (Kissel et al., 1991; Nikolaos, Kelley, Madeline, & Anthony, 1999), orthopedic devices (Middleton, & Tipton, 2000), sutures, cardiac pacemakers, and vascular grafts. Natural polymers such as konjac glucomannan (Ying-qing, Bi-jun, & Xin, 2005), chitosan (Carreno-Gomez & Duncan, 1997; Takashi et al., 1997; Ding et al., 2004), and gelatin (DiSilvio, & Downes, 1997; Lopes & Felisberti, 2003; Draye, et al., 1998) have remained attractive primarily because they are economical, readily available, and potentially degradable and compatible due to their origin.

Konjac glucomannan is a heteropolysaccharide derived from the konjac tuber. It consists of 1,4-linked β-D-mannopyranose and β-D-glucopyranose units in a molar ratio of 1.6:1 with a low degree of acetyl groups at the side chain C-6 position and having a molecular weight on average of 0.67–1.9 million (Li & Xie, 2004). It has been generally used in food, film-formation, chemical engineering, and also specifically in biomedical applications, for example, drug delivery (Wang & He, 2002; Pathak, & Barman, 2003), cellular therapy (Slepian & Massia, 2001), etc.

Chitosan is the most abundant natural polysaccharides containing nitrogen. It is the *N*-deacetylated derivative of chitin, a cationic polysaccharide composed of β-D-glucosamine

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and *N*-acetyl-β-D-glucosamine residues with 1,4 linkages (Majeti & Kumar, 2000; Xiao, Gao, Wang, & Zhang, 2000). Chitosan and its derivatives have been identified as hydrophilic, non-toxic, biodegradable, antibacterial materials suitable for tissue engineering as the scaffold (Madihally, & Matthew, 1999; Zhang, & Zhang, 2001; Lee, et al., 2002; Shanmugasundaram, et al., 2001). It has been used as artificial skin to accelerate wound and ulcer healing and as a biocompatible vehicle for sustained release of drug (Oungbho, & Müller, 1997). The positive surface charge and biocompatibility of chitosan enabled it effectively to support cell growth (Zhang, Li, Gong, Zhao, & Zhang, 2002).

However, chitosan also has some drawbacks, it being soluble in aqueous medium only in the presence of a small amount of acid. Its mechanical properties have also proved to be unsuitable in some biomedical applications (Chuang, Young, Yao, & Chiu, 1999; Hu, Jou, & Yang, 2004).

This paper reports work aimed at modifying chitosan by blending it with konjac glucumannan to improve the mechanical properties and water solubility and the biocompatibility of chitosan as a konjac glucomannan/chitosan blend film. The morphological structure, thermal stability, and mechanical properties of the blend films were studied by infrared (IR), wide-angle X-ray diffraction (WAXD), differential scanning calorimetry (DSC), scanning electron microscope (SEM), and electron tensile test. The relationship between the structure and their physicochemical properties is discussed. In addition, the work also sought to find out whether a konjac glucomannan/chitosan blend film would have the desired biocompatibility according to the MTT (3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide; Thiazolyl blue) assay and cell morphological observation.

2. Materials and methods

2.1. Materials

Konjac glucomannan was extracted and purified from the tuber of Konjac *Amorphophallus*. The tubers were sliced to about 8 mm in thickness and then dried under heated air at 65 °C for about 6 h. The dried sheets were pulverized by mill. The crude flour was immersed in 50% (v/v) aqueous methanol for 3 h and then dried at 50 °C under reduced pressure. Then, the raw konjac flour was extracted with benzene–absolute alcohol (4:1 v/v) and trichloromethane—*n*-butanol (Sevag method) for five times, respectively. The fat- and protein-extracted flour was dissolved with a mixture of distilled water/hydrogen peroxide [H_2O: 30% H_2O_2 = 5:1 (v/v)] and heated at 40 °C for 20 min; After environmental cooling to room temperature, the hydrosol was centrifuged at 17,000*g* for 20 min (Himac Centrifuge, Hitachi). Then, acetone was added to the supernatant and stirred. After being filtered with a 128 μm mesh sieve, the white cotton-like precipitate was squashed, and then dried by vacuum freeze drying (Li & Xie, 2004). This konjac glucomannan had a viscosity-average molecular weight (Mv) as determined by viscometry of 9.89×10^6 according to the Mark–Houwink equation $[\eta]=5.96\times10^2 Mv^{0.73}$ at 25 °C.

The chitosan was purchased from Wuhan Tianyuan biomaterial Co. (Wuhan, China). Its degree of deacetylation was measured to be 85% by the method of Tingda Jiang (2002), and the viscosity-average molecular weight (Mv) of the chitosan was determined by viscometry and result was 1.68×10^6 according to the Mark–Houwink equation $[\eta]=1.424\times10^{-3}\ Mv^{0.96}$ at 25 °C.

2.2. Preparation of blend films

Purified totally soluble konjac glucomannan was dissolved in distilled water to a concentration of 1 wt%. Chitosan was dissolved in a 0.8 wt% aqueous sodium to prepare a concentration of 1 wt% solution. The solutions of konjac glucomannan and chitosan with different mixing ratios [90/10, 80/20, 60/40, 40/60 and 20/80 konjac glucomannan/chitosan (w/w)] were cast onto polystyrene plates and dried at room temperature. A series of blend films were there by obtained and coded as KC1, KC2, KC4, KC6, and KC8. The films obtained from pure konjac glucomannan and chitosan was coded as KGM and CHI.

2.3. Characterization of films

The powdered films were blended with potassium bromide and laminated, and the IR spectra were recorded with a Nicolet (USA) Nexus 470 FTIR spectrometer. Film samples of about 100 μm thickness were coated with gold in 0.1 T vacuum degree; The cross-section morphologies were observed on a Hitachi X-650 SEM. The X-ray diffraction (XRD) curves of the films were recorded with a Rigaku (Japan) D/max-RB X-ray diffractometer and used a Cu Kα target at 40 kV and 50 mA. The diffraction angle ranged from 60 to 5°. The crystallinities of the films were calculated by $X_c=(F_c/(F_c+F_a))100\%$, where F_c and F_a are the areas of crystal and non-crystalline regions, respectively. The DSC of the film samples (10 mg) was performed under a nitrogen atmosphere with a DSC 200PC (NETZSCH, Germany) with a flow capacity of 25 ml/min from 30 to 400 °C at a heating rate of 10 °C/min. The tensile strength (σ_b) and breaking elongation (ε_b) of the films were measured on an electron tensile tester CMT-6104 (Shenzhen Sans Test Machine Co., Ltd, China) with a tensile rate of 250 mm/min according to the Chinese standard method (GB/T4456-96).

2.4. Cell culture

L929 fibroblast cells (obtained from Tongji Medical College of Huazhong University of Science and Technology, Wuhan, China) were cultured in RPMI medium 1640 containing 10% fetal bovine serum and 100 mg/ml penicillin and 100 mg/ml streptomycin. They were incubated in a 37 °C water-jacketed incubator equilibrated with 5% CO_2 and kept at approximately 99% relative humidity; the culture medium was changed every 3 days. At confluence, the fibroblast cells were harvested and subcultivated in the same medium.

2.5. Extraction method

The KC2 blend film was soaked in serum-supplemented culture medium (RPMI 1640) under 6 cm^2/ml of culture medium for 72 h at 37 °C according to the Chinese standard method, the supernatant was extracted carefully, neutralized to exclude the effect of pH and filtered through a 0.2 μm film filter (GB/T16886.5-97). So the extracts that contained almost all the soluble compounds of the KC2 blend film in culture medium was obtained. Then the extracts were diluted with culture medium, a series of the extract dilutions (50, 25, 12.5, and 6.25% (v/v)) was collected. The cells were seeded into 96-well plates at a density of 1×105 cells/ml. The culture medium (100 μl) was replaced with each concentration extract dilutions after 24 h. After 24, 48, and 72 h incubation, the viability of every group was assessed by the MTT assay (see below). Serum-supplemented culture medium (RPMI 1640) was used as control groups.

2.6. MTT assay

The cytotoxicity of KC2 extracts was evaluated against L929 cells by using the methyl tetrazolium (MTT) assay in a 96-well plate method (Eick, et al., 2002). MTT (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) was dissolved in phosphate-buffered saline which was prepared with NaCl 8.00 g, KCl 0.20 g, $NaHPO_4$ 1.15 g, KH_2PO_4 0.20 g, H_2O 1000 ml (0.1 mol/l pH 7.2) at 5 mg/ml and filtered for sterilization. MTT in PBS (25 μl) was added to each well of 96-well plates. After 4 h incubation at 37 °C, unreacted dye was removed by aspiration and the purple formazan product was dissolved in dimethyl sulfoxide (150 μl/well) and measured spectrophotometrically using an enzyme-linked immunosorbent assay (ELISA) Reader (MODEL550, BIO-RAD, USA) at 490 nm (OD490). The spectrophotometer was calibrated using culture medium without cells. The relative cell growth (%) related to control cells containing cell culture medium without extracts was calculated from the following equation:

$$\text{Relative cell growth (\%)} = \frac{[\text{OD}]_{\text{text}}}{[\text{OD}]_{\text{control}}} 100\%$$

2.7. Light microscopy

The solution or mixed solution under test was spread on coverslips and dried at room temperature, and sterilized for 30 min by UV irradiation. They were cast onto the 6-well plate. The cells were seeded into the 6-well plates at a density of 1×10^5 cells/ml. The growth of fibroblasts on blend films was observed and photographed under an inverted microscope (Olympus, Tokyo, Japan) after 48 h.

The growth of fibroblasts on blend films was observed and photographed under an inverted microscope (Olympus, Tokyo, Japan) every day. Photographs were taken every 24 h. Pristine glass surfaces were used as control groups.

3. Results and discussion

3.1. Structure and miscibility

In the IR spectra of the films of KC1, KC2, KC4, KC6, and KC8, KGM and CHI (Fig. 1), the absorption band at 3441 cm^{-1} and the peaks at 2924 cm^{-1} were assigned to the stretching of –OH groups and C–H of methyl in konjac

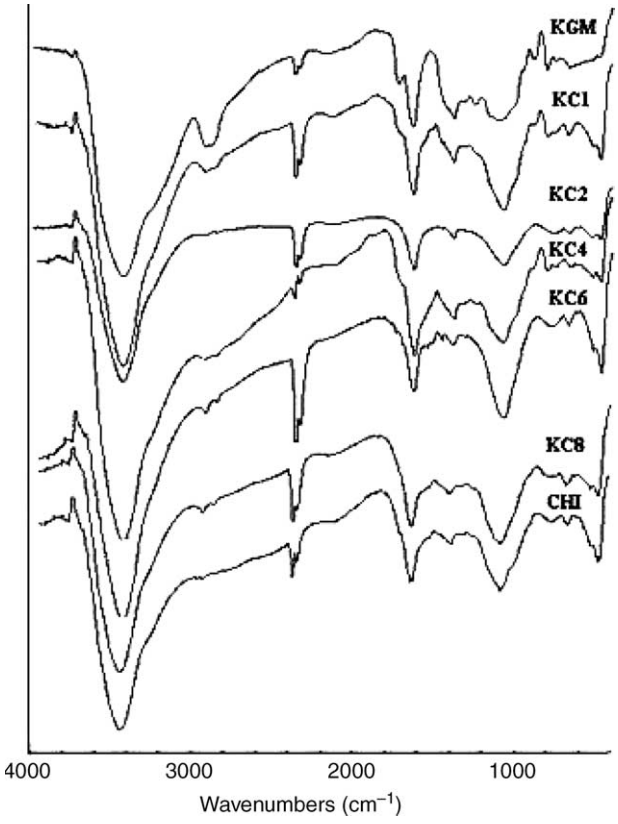

Fig. 1. FTIR spectra of the KC1, KC2, KC4, KC6, KC8, KGM and CHI films.

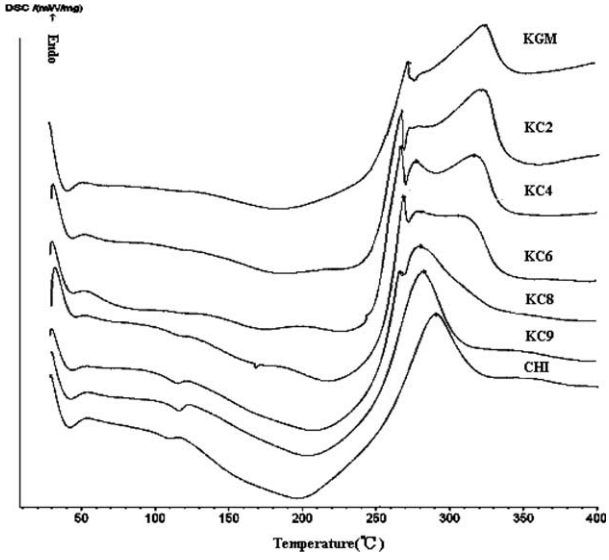

Fig. 2. The DSC curves of the KC1, KC2, KC4, KC6, KC8, KGM and CHI films.

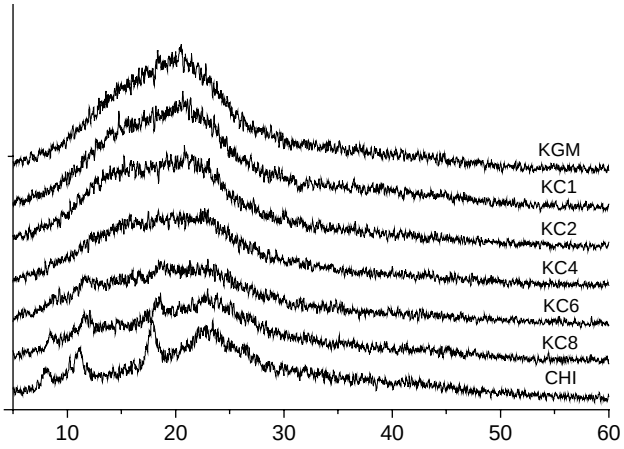

Fig. 3. WXRD diffraction curves of the KC1, KC2, KC4, KC6, KC8, KGM and CHI films.

glucomannan; the characteristic absorption bands of mannose in konjac glucomannan appeared at 886 and 804 cm^{-1}. The broad peak at 1638 cm^{-1} was assigned to stretching of C–O of associate hydroxy groups. The absorption band at 3445 cm^{-1} was assigned to the stretching of N–H groups bonded to –OH in chitosan; the peaks at 1371 cm^{-1} ware assigned to the characteristic bending absorption band of amino groups and the stretching of amide III; the peaks at 1077 cm^{-1} were assigned to the characteristic absorption band of C_6–OH. Compared with the spectrum of (pure) KGM and CHI, the following changes had taken place in the blend films. The absorption band around 3440 cm^{-1} broadened and shifted to a lower wave number with the increase of konjac glucomannan, indicating the gradual increase of intermolecular hydrogen bonds between chitosan and konjac glucomannan. The stretching of carbonyl at 1723 cm^{-1} of konjac glucomannan disappeared; and the stretching of intramolecular hydrogen bonds at 1638 cm^{-1} in konjac glucomannan coupled and shifted to a lower wave number, suggesting that the new hydrogen bonds between chitosan and konjac glucomannan molecules in the blend films occurred.

The DSC curves of the KGM, KC1, KC2, KC4, KC6, KC8, and CHI films (Fig. 2) were used to determine the compatibility of the blend by means of testing the endothermic peaks. In the CHI curve, the main feature was that there was an endothermic peak at 283 °C. The presence of such an endothermic peak could not be attributed to the melting point of chitosan since a recrystallization peak did not occur on subsequent cooling. Hence, this peak, evident in the DSC traces, essentially characterizes the thermal behavior of chitosan. The presence of this peak presumably resulted from the thermal decomposition of the glycosidic bonds of chitosan. Similarly, KGM revealed the decomposition peaks at 266 and 320 °C. The DSC curve of the konjac glucomannan/chitosan blend films exhibited the thermal properties of the two main polymers: the decomposition temperature of the konjac glucomannan, and the decomposition temperature of chitosan which shifted to a temperature 10 °C lower than in the pure chitosan. In addition, the difference of the decomposition temperature of konjac glucomannan and chitosan was least in the KC2 curve. In general, the glass-transition temperature (T_g) of blend films was difficult to detect by using the ordinary DSC technique; in this paper, we did not give the exact value of T_g, but the changes of number and location of the endothermic peaks in DSC curve indicated that konjac glucomannan and chitosan

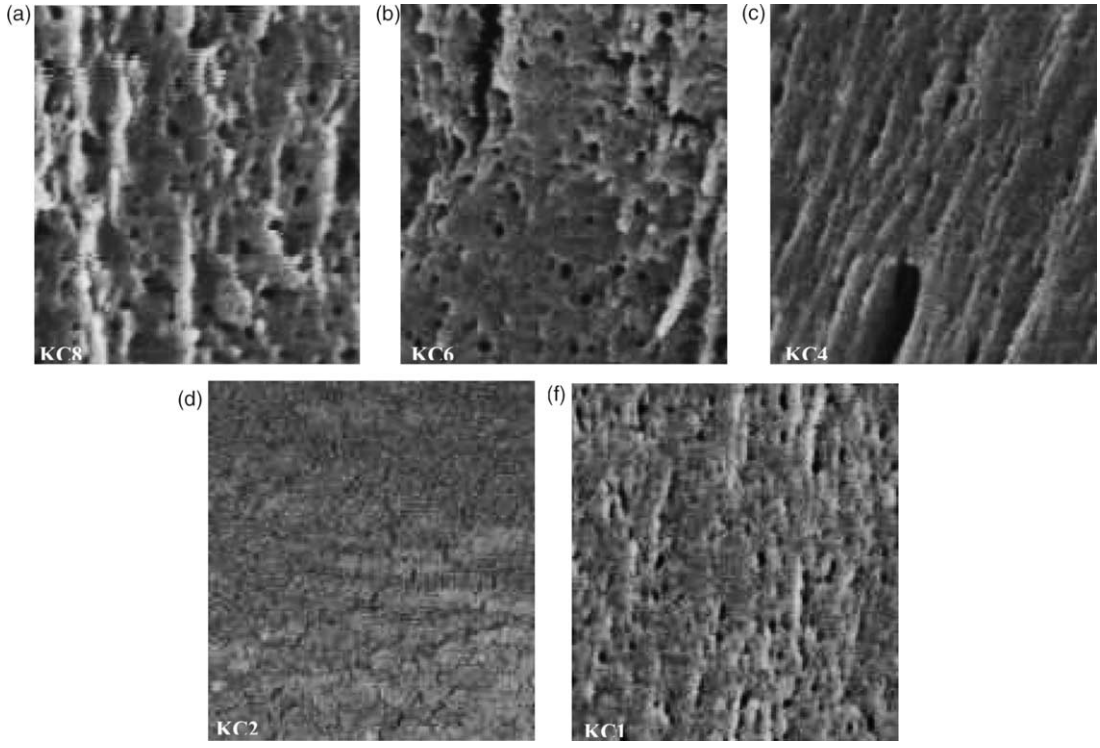

Fig. 4. SEM photographs of the cross-section for KC1, KC2, KC4, KC6 and KC8 films.

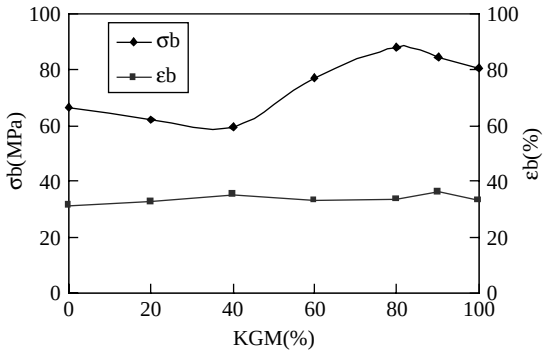

Fig. 5. Tensile strength (σ_b) and breaking elongation (ε_b) dependence of the content of KGM film.

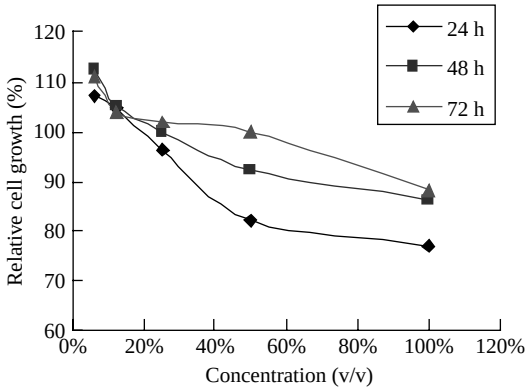

Fig. 6. Effects of the concentration of the extracts of KC2 on the relative cell growth of L929 cells after 24, 48 and 72 h.

form a hydrogen-bonding interaction between them in the blend films.

The WXRD curves of KGM, KC1, KC2, KC4, KC6, KC8, and CHI films are shown in Fig. 3. The crystallinities of KGM, KC1, KC2, KC4, KC6, KC8, and CHI were 48.9, 45.9, 31.5, 49.3, 45.7, 42.1 and 37.6%, respectively. Four crystal peaks could be recognized at around $2\theta = 7.8$, 11.1, 17.8 and 23.4° in the XRD pattern of CHI. The pure KGM film showed a non-crystalline state and only had a very broad peak around $2\theta = 20.0°$. With the increase of the content of konjac glucomannan, the diffraction peak corresponding to 11.1° of chitosan became gradually lowered, and the diffraction angle neared more and more to 20.0° accordingly. The same regularity could be drawn in regard to diffraction peak around $2\theta = 7.8$, 17.8 and 23.4°. The diffraction peaks of KGM and CHI drew closer to each so it indicated that the interaction between KGM and CHI increased with the increase of konjac glucomanan content.

SEM is an important method for characterization of the miscibility of two or more polymers. The compatibility of two kinds of high polymer could be evaluated from the degrees of homogeneity and the compactness has of the blend film, i.e. the blend would be homogeneous and compact when the two kinds of high polymer had a good miscibility. The scanning electron micrographs of the films were shown in Fig. 4. The cross-section morphologies of the film CH8 were an obvious phase separation, the phase separation decreased again with the increase of konjac glucomannan content. When the ratio of konjac glucomannan to chitosan was 80:20 by weight, the blend film KC2 showed smooth and homogeneous cross-section morphology, suggesting a high miscibility and blend homogeneity between the chitosan and konjac glucomannan.

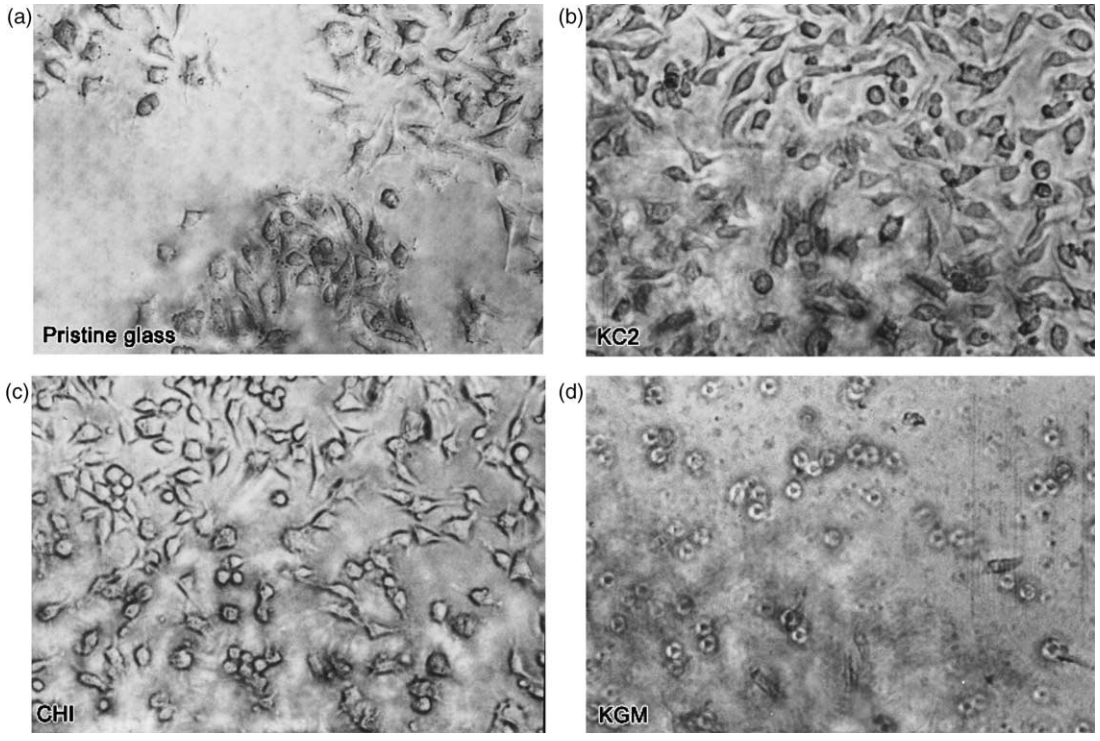

Fig. 7. Photographs of L929 cells cultured for 48 h on KGM, CHI, KC2 and pristine glass (light micrograph, ×100).

3.2. Mechanical properties

The study of mechanical properties is of primary importance for determining the performance of materials, especially that of film materials. The tensile strength (σ_b) and breaking elongation (ε_b) dependence of the konjac glucomannan content are shown in Fig. 5. The tensile strengths of the blend films increased with the increase of konjac glucomannan content, and the maximum value appeared at the KGM/CHI = 80/20 mixing ratio (KC2) and achieved 88.05 MPa. The breaking elongation (ε_b) of the film was about 33% of average. The considerable enhancement in tensile strength of blend films indicated that intermolecular interactions between the konjac glucomannan and chitosan in them.

3.3. Biocompatibility of the KC2 blend film

MTT reagent is a pale yellow substrate, which produces a dark blue formazan product when incubated with viable cells. Therefore, the level of the reduction of MTT into formazan could reflect the level of cell metabolism. Fig. 6 shows effects of the concentration of the extracts on the relative cell growth after 24, 48 and 72 h. The relative cell growth of extracts and dilutions were more than 75% after 24, 48 and 72 h. The relative cell growth increased with the decrease of the concentration of extracts, and fibroblasts proliferated in low concentration extracts relative to the control. In addition, the relative cell growth increased with the increase of days.

Photographs of the mouse fibroblast L929 cells cultured for 48 h on the various materials are shown in Fig. 7. The surfaces of the KGM, CHI and KC2 films have different effects on the fibroblasts. Only a fraction of cells could attach on to the KGM membrane. In contrast, when the films were blended by chitosan, they became substrates with properties much like those of controls. The cells attached and spread on the KC2 blend film surfaces without apparent impairment of cell morphology.

From the MTT assay and cell morphology evaluation, it was confirmed that the KC2 blend films had good biocompatibility and in this respect was the best of the films produced.

4. Conclusions

A series of transparent blend films, prepared by blending 1% w/v aqueous konjac glucomannan with 1% w/v chitosan in acetate solution according to predetermined ratios and drying at 40 °C on polystyrene plates for 4 h, all exhibited mechanical properties in dry state that were obviously higher than those of chitosan film. The mechanical properties achieved their maximum when the weight ratio of konjac glucomannan to chitosan was 8:2. FTIR, SEM, WAXD and DSC proved good miscibility between chitosan and konjac glucomannan in KC2 blend film. The strong intermolecular hydrogen bonds existing between the amino groups of chitosan and the hydroxyl groups of konjac glucomannan in the blend films resulted in the enhancement of mechanical properties. In addition, the MTT assay and cell morphology evaluation proved that the KC2 blend films had good biocompatibility, making it possible to apply the KC2 blend film to biomaterial.

5. Uncited references

Biological evaluation of medical devices. Khor and Lim, 2003. Polyethylene blown film for packaging. Yuan et al., 2004.

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References

Biological evaluation of medical devices: Tests for cytotoxicity: In vitro methods. GB/T16886.5-1997 (Chinese).

Carreno-Gomez, Begona, & Duncan, Ruth (1997). Evaluation of the biological properties of soluble chitosan and chitosan microspheres. *International Journal of Pharmaceutics*, *148*, 231–240.

Chuang, Wen-yuan, Young, Tai-horng, Yao, Chun-Hsu, & Chiu, Wen-Yen (1999). Properties of the poly(vinyl alcohol)/chitosan blend and its effect on the culture of fibroblast in vitro. *Biomaterials*, *20*, 1479–1487.

Ding, Zhi, Chen, Jiangning, Gao, Shuying, Chang, Jianbing, Zhang, Junfeng, & Kang, E. T. (2004). Immobilization of chitosan onto poly-L-lactic acid film surface by plasma graft polymerization to control the morphology of fibroblast and liver cells. *Biomaterials*, *25*, 1059–1067.

DiSilvio, L., & Downes, S. (1997). Biodegradable gelatin microspheres for site-specific delivery of polypeptides. *Journal of Controlled Release*, *46*, 185.

Draye, Jean-Pierre, Delaey, Bernard, Van de Voorde, Andre, Van Den Bulcke, An, De Reu, Bepke, & Schacht, Etienne (1998). In vitro and in vivo biocompatibility of dextran dialdehyde cross-linked gelatin hydrogel films. *Biomaterials*, *19*, 1677–1687.

Eick, J. D., Kostoryz, E. L., Rozzi, S. M., Jacobs, D. W., Oxman, J. D., Chappelow, C. C., et al. (2002). In vitro biocompatibility of oxirane/polyol dental composites with promising physical properties. *Dental Materials*, *18*, 413–421.

Hu, S. G., Jou, C. H., & Yang, M. C. (2004). Biocompatibility and antibacterial activity of chitosan and collagen immobilized poly(3-hydroxybutyric acid-*co*-3-hydroxyvaleric acid). *Carbohydrate Polymers*, *58*, 173–179.

Jiang, Tingda (2002). *Chitosan*. Beijing: Chemical Industry Press (in Chinese).

Khor, Eugene, & Lim, Lee Yong (2003). Implantable applications of chitin and chitosan. *Biomaterials*, *24*, 2339–2349.

Kissel, T., Birch, Z., Bantle, S., Lancranjan, I., Nimmerfall, F., & Vit, F. (1991). Parenteral depot systems on the basis of biodegradable polymers. *Journal of Controlled Release*, *16*, 27–42.

Lee, Jue-Yeon, Nam, S. H., Im, S. Y., Park, Y. J., Lee, Y. M., Seol, Y. J., et al. (2002). Enhanced bone formation by controlled growth factor delivery from chitosan-based biomaterials. *Journal of Controlled Release*, *78*, 187–197.

Li, Bin, & Xie, Bijun (2004). Synthesis and characterization of konjac glucomannan/poly(vinyl alcohol) interpenetrating polymer networks. *Journal of Applied Polymer Science*, *93*, 2775–2780.

Lopes, Cristina M.A., & Felisberti, Maria I. (2003). Mechanical behaviour and biocompatibility of poly(1-vinyl-2-pyrrolidinone)-gelatin IPN hydrogels. *Biomaterials*, *24*, 1279–1284.

Madihally, Sundararajan V., & Matthew, Howard W.T. (1999). Porous chitosan scaffolds for tissue engineering. *Biomaterials*, *20*, 1133–1142.

Majeti, N. V., & Kumar, Ravi (2000). A review of chitin and chitosan applications. *Reactive and Functional Polymers*, *46*, 1–27.

Middleton, John C., & Tipton, Arthur J. (2000). Synthetic biodegradable polymers as orthopedic devices. *Biomaterials*, *21*, 2335–2346.

Mori, Takashi, Okumura, M., Matsuura, M., Ueno, K., Tokura, S., Okamoto, Y., et al. (1997). Effects of chitin and its derivatives on the proliferation and cytokine production of fibroblasts in vitro. *Biomaterials*, *18*, 947–951.

Oungbho, Kwunchit, & Müller, Bernd W. (1997). Chitosan sponges as sustained release drug carriers. *International Journal of Pharmaceutics*, *156*, 229–237.

Pathak, C. P., & Barman, S. P. (2003). Multiblock biodegradable hydrogels for drug delivery and tissue treatment. United States Patent, 6,639,014.

Peppas, Nikolaos A., Keys, Kelley B., Torres-Lugo, Madeline, & Lowman, Anthony (1999). Poly(ethylene glycol)-containing hydrogels in drug delivery. *Journal of Controlled Release*, *62*, 81–87.

Polyethylene blown film for packaging, *GB/T 4456-1996* (Chinese).

Serrano, M. C., Pagani, R., Vallet-Regi, M., Pena, J., Ramila, A., Izquierdo, I., et al. (2004). In vitro biocompatibility assessment of poly(ε-caprolactone) films using L929 mouse fibroblasts. *Biomaterials*, *25*, 5603–5611.

Shanmugasundaram, N., Ravichandran, P., Neelakanta, P., Ramamurty, Nalini, Pal, Subrata, & Rao, Panduranga (2001). Collagen–chitosan polymeric scaffolds for the in vitro culture of human epidermoid carcinoma cells. *Biomaterials*, *22*, 1943–1951.

Slepian, M. J., & Massia, S. P. (2001). *Local polymeric gel cellular therapy*. United States Patent, 6,290,729.

Wang, Kang, & He, Zhimin (2002). Alginate-konjac glucomannan–chitosan beads as controlled release matrix. *International Journal of Pharmaceutics*, *244*, 117–126.

Xiao, Chaobo, Gao, Shanjun, Wang, Heng, & Zhang, Lina (2000). Blend films from chitosan and konjac glucomannan solutions. *Journal of Applied Polymer Science*, *76*, 509–515.

Yuan, Ying, Zhang, Peiyun, Yang, Yuming, Wang, Xiaodong, & Gu, Xiaosong (2004). The interaction of Schwann cells with chitosan membranes and fibers in vitro. *Biomaterials*, *25*, 4273–4278.

Zhang, M., Li, X. H., Gong, Y. D., Zhao, N. M., & Zhang, X. F. (2002). Properties and biocompatibility of chitosan films modified by blending with PEG. *Biomaterials*, *23*, 2641–2648.

Zhang, Ying-qing, Xie, Bi-jun, & Gan, Xin (2005). Advance in the applications of konjac glucomannan and its derivatives. *Carbohydrate Polymers*, *60*, 27–31.

Zhang, Yong, & Zhang, Miqin (2001). Microstructural and mechanical characterization of chitosan scaffolds reinforced by calcium phosphates. *Journal of Non-Crystalline Solids*, *282*, 159–164.