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Investigation of PVA/ws-chitosan hydrogels prepared by combined γ-irradiation and freeze-thawing

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Abstract

γ-Irradiation combined with freeze-thawing, i.e. irradiation followed by freeze-thawing and freeze-thawing followed by irradiation, was applied to prepare poly(vinyl alcohol) (PVA)/water soluble chitosan (ws-chitosan) hydrogels for wound dressing. The properties of these hydrogels were investigated and compared to those prepared by freeze-thawing and by irradiation, respectively. Hydrogels made by irradiation followed by freeze-thawing show larger swelling capacity and mechanical strength, higher thermal stability, lower water evaporation rate, and are less turbid than those made by pure freeze-thawing and freeze-thawing followed by irradiation. Hydrogels made by irradiation alone cannot be used as wound dressing due to their poor mechanical strength. SEM results show that the final structure of hydrogels made by combined irradiation and freeze-thawing is mainly determined by the first processing step. It is found that the appropriate amount of ws-chitosan can endow hydrogels with large swelling capacity and mechanical strength. The presence of ws-chitosan provides the hydrogels with good antibacterial activity against *Escherichia coli (E. coli)*.

Keywords: Hydrogels; Freeze-thawing; Irradiation; Rheology; Water soluble chitosan

1. Introduction

Hydrogels are cross-linked hydrophilic polymer networks which can absorb large amounts of water without dissolution. Owing to their similar physical properties to human tissues and their excellent tissue compatibility, hydrogels have been studied extensively for biomedical applications. They can be used as soft contact lenses (Opdahl, Kim, Koffas, Marmo, & Somorjai, 2003), tissue engineering scaffolds (Nowak et al., 2002), controlled drug-release vehicles (Qiu & Park, 2001) and wound dressings (Sen & Avci, 2005; Wu et al., 2004; Yang & Lin, 2004). Hydrogels have many advantages as wound dressings. For instance, they can absorb excess of wound exudates, protect the wound from secondary infection and effectively promote the healing process by providing a moisturized wound healing environment (Winter, 1962). They can also be removed without causing trauma to the wound.

Chitosan, the partially deacetylated form of chitin, is a well known material in the wound dressing field. It has excellent biocompatibility, biodegradability, hemostatic, and antibacterial activity. In general, chitosan with a high molecular weight is insoluble in water but can dissolve in acid solution. Hydrogels made from chitosan acid solution often need a repeated washing process to neutralize the acid. The use of water-soluble chitosan (ws-chitosan) can simplify the process of making hydrogels. Poly (vinyl alcohol) (PVA) is a water-soluble polyhydroxy polymer. It has been used in practical applications because of its easy preparation, excellent chemical resistance and physical properties, and it is completely biodegradable and cheap. With these considerations, the blends of ws-chitosan and PVA were used as the hydrogel materials for wound dressing in this experiment.

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Hydrogels can be made by irradiation, freeze-thawing or chemical methods. Irradiation is considered as a suitable tool for the formation of hydrogels. It has the advantages of easy control of processing, no necessity to add any initiators or cross-linkers which may be harmful and difficult to remove, and possesses the possibility of combining hydrogel formation and sterilization in one technological step. The main disadvantage of hydrogels prepared by irradiation is their poor mechanical strength. Hydrogels prepared by freeze-thawing from PVA aqueous solutions have shown many interesting properties. They have good mechanical strength, are stable at room temperature, and with no initiators or cross-linkers. The main disadvantages of this kind of hydrogel are its opaque appearance and the limited swelling capacity and thermal stability.

Up to now, much work has been done on preparing hydrogels by irradiation (Lopergolo, Lugao, & Catalaini, 2002; Park & Nho, 2004; Sen & Avci, 2005; Zhao, Mitomo, Nagasawa, Yoshii, & Kume, 2003a) or by freeze-thawing (Bajpai & Saini, 2005; Hickey & Peppas, 1997; Nugent, Hanley, Tomkins, & Higginbotham, 2005; Ricciardi, Gaillet, Ducouret, Lafuma, & Laupretre, 2003). However, there is very little information on preparation of hydrogels by combining the two processing techniques (Nho & Park, 2002), especially by irradiation followed by freeze-thawing. In this research, PVA hydrogels with or without ws-chitosan were prepared by combined γ-irradiation and freeze-thawing, namely irradiation followed by freeze-thawing and freeze-thawing followed by irradiation. The properties of these hydrogels including the gel fraction, the swelling behavior, the water evaporation rate, the rheological properties, and the antibacterial behavior, as well as their relationship to ws-chitosan content were investigated. The results were discussed in comparison with hydrogels prepared by pure irradiation and pure freeze-thawing.

2. Experimental

2.1. Materials

PVA-1750 (M_w: 80 500) was bought from Sinopharm Chemical Reagent Co. Ltd., China. Ws-chitosan, manufactured by protonation of chitosan in HCl/CH_3CH_2OH solution, was obtained from Jinhu Chitin Co. Ltd., China. The weight-average molecular weight and deacetylation degree of the chitosan before protonation were 200 000 and 91.7%, respectively. Agar (Bacteriological Grade) was bought from Xiamen Xinglongda Chemical Reagent Co. Ltd., China. Nutrient broth was purchased from Shanghai Kangrun Biotech Co. Ltd., China.

2.2. Preparation of the hydrogels

Aqueous solutions of PVA were obtained by dissolving PVA in distilled water at 96 °C under refluxing for 3 h. The solution was mixed with ws-chitosan solutions at 45 °C and stirred for 30 min with a physical stirrer. In order to remove bubbles, the solutions were placed in an ultra sonic water bath at 45 °C for 15 min. The aqueous solutions with 7 wt% PVA and various contents of ws-chitosan were then poured into Petri dishes. Hydrogels were obtained by γ-irradiation (Irra.), γ-irradiation followed by freeze-thawing (Irra. + FT), freeze-thawing (FT), and freeze-thawing followed by γ-irradiation (FT+Irra.), respectively. Irradiation was performed in N_2 atmosphere with ^{60}Co γ-ray to a dose of 30 kGy and at a dose rate of 0.76 kGy/h. Freezing and thawing were repeated up to three times to form hydrogels. Each cycle of freeze-thawing involved lowering the temperature to −20 °C, standing at this temperature for 1.5 h, and then raising the temperature to 25 °C, standing at this temperature for 1 h.

2.3. Gel fraction

Hydrogels made by various methods were first dried at 60 °C for 48 h until a constant weight (W_d) was reached. Then the sol part was extracted by immersing the dried gels in hot distilled water at 45 °C for 72 h with water being changed every 5 h. The extracted hydrogels were dried at 60 °C for 48 h to a constant weight (W_r). The gel fraction was defined as $\text{Gel}\ (\%) = (W_r/W_d) \times 100$.

2.4. Swelling behavior

Hydrogels made by various methods were extracted in distilled water at 45 °C for 72 h. After the water on the surface of the extracted gels was removed with cellulose paper, the gels were immersed in various buffer solutions (0.01 mol/L) for at least 24 h until an equilibrium state of swelling (with a weight of W_s) was achieved. Then the swollen gels were dried at 60 °C for 48 h to a constant weight (W_d). The degree of swelling (DS) of gels in various buffer solutions was calculated as $DS = W_s/W_d$.

The swelling kinetics of hydrogels in distilled water at 37 °C was followed by measuring the weight (W_t) of hydrogels at regular intervals until the gels reached the equilibrium state of swelling (weight = W_s). The swelling kinetics of hydrogels was calculated by the following equation

$$\text{Water retained}(\%) = (W_t - W_d)/(W_s - W_d) \times 100$$

where W_d was the weight of the initially dried gels.

2.5. Evaporation rate of water from hydrogel

The swollen hydrogels (with a weight of W_s) were kept at 37 °C and 40% relative humidity in an incubator. After regular intervals of time, the weight (W_t) was measured. Water loss percentage was calculated by the following equation

$$\text{Water lost}(\%) = (W_s - W_t)/(W_s - W_d) \times 100$$

where W_d was the weight when the gel lost all its water.

2.6. Morphology observation

The morphology of the hydrogels after being freeze-dried was studied by scanning electron microscopy (SEM, 1530VP, LEO Corporation). Prior to examination, the samples were fractured in liquid nitrogen, and the fractured surface was coated with gold.

2.7. Rheological measurements

The rheological measurements were conducted on a strain controlled ARES rheometer (TA Instruments) using parallel plates of 25 mm in diameter with plate-to-plate distance of 1–2 mm. All rheological measurements were carried out at a small strain (0.2%) which ensured that the deformation imposed on the gel was entirely reversible. The experiment went through frequency scanning at 25 °C in the frequency range of 0.2–100 rad/s. The temperature dependence of the rheological properties was investigated in the temperature range of 25 to 100 °C at a shear frequency of 1 rad/s with a ramp rate of 5 °C/min. To prevent dehydration during rheological measurements, a thin layer of vaseline was placed on the peripheral surface of the hydrogel held between the plates. Each measurement was performed at least twice, on two different disc specimens from the same hydrogel sample.

2.8. Antibacterial assessment

Antibacterial activity of PVA hydrogels with or without ws-chitosan against *Escherichia coli (E. coli)* was evaluated by a solid medium method. First, 0.5 g of hydrogel and agar were added to 15 mL media (Nutrient broth) and the mixture were kept at 40 °C for 48 h. The mixtures were then sterilized at 121 °C for 20 min in which process agar could be dissolved. The sterilized solutions were poured into 90-mm petri dishes. Hundred microlitres of decimal dilution of *E. coli* culture, which had been incubated at 37 °C for 12 h, was added to the media plates after cooling the plates down to the room temperature. The media plates were then incubated at 37 °C for 48 h. The antibacterial property of hydrogels can be determined by observing the quantity of *E. coli* colonies on the media plates. The media plate without any hydrogel was used as control. All of the operations were done under aseptic condition or by an aseptic technique.

3. Results and discussion

3.1. Appearance of the hydrogels

The appearance of hydrogels synthesized by various methods differs significantly. As shown in Fig. 1, hydrogels prepared by pure freeze-thawing and freeze-thawing followed by irradiation are opaque while those made by irradiation followed by freeze-thawing and by pure irradiation are translucent and transparent, respectively. It has been reported that the opaque nature of gels prepared by pure freeze-thawing is attributed to the microphase separation which occurs in the early stage of the gelation process (Nugent et al., 2005; Ricciardi et al., 2003; Takeshita, Kanaya, Nishida, & Kaji, 1999), while the transparent appearance of hydrogels made by irradiation is attributed to the homogeneous network structure (Gu & Zhu, 2005). The translucent nature of hydrogels made by irradiation followed by freeze-thawing is likely due to the chemical cross-linking of polymer chains induced by irradiation, which reduces the phase separation in the following freeze-thawing process.

3.2. Gel fraction

The gel fractions of PVA/ws-chitosan hydrogels made by various methods are compared in Fig. 2. The gel frac-

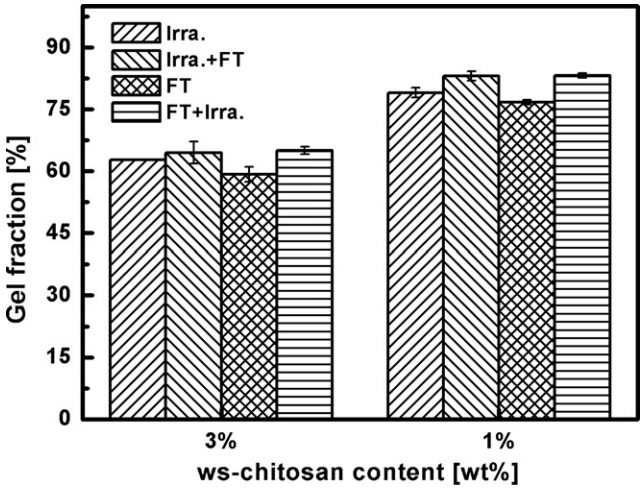

Fig. 2. Gel fraction of PVA/ws-chitosan hydrogels with different amount of ws-chitosan made by various methods. The content of PVA is 7 wt%.

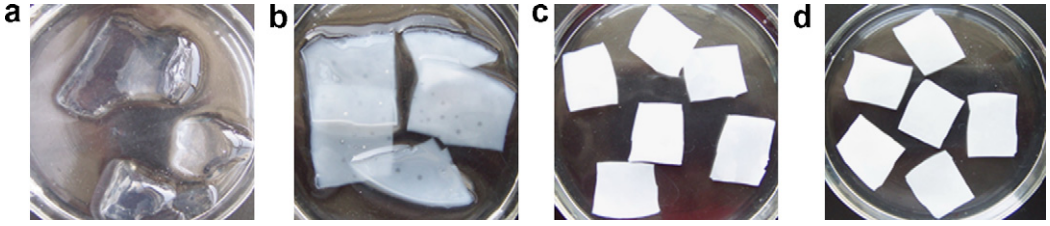

Fig. 1. The morphologies of hydrogels made by (a) pure irradiation, (b) irradiation followed by freeze-thawing, (c) pure freeze-thawing, and (d) freeze-thawing followed by irradiation. The composition of the hydrogels is 7 wt% PVA and 3 wt% ws-chitosan.

tion decreases rapidly with increasing ws-chitosan content. This can be explained by the lower cross-linking density in the presence of more ws-chitosan. Some researchers have reported that polysaccharide derivatives such as carboxymethylcellulose (CMC), carboxymethylchitin (CM-chitin) and carboxymethylchitosan (CM-chitosan) aqueous solutions cannot be cross-linked through irradiation if the content of the polymer is very low (below 10 wt%) (Fei, Wach, Mitomo, Yoshii, & Kume, 2000; Wach, Mitomo, Yoshii, & Kume, 2001; Zhao et al., 2003a, 2003b). Hydrogels with more content of ws-chitosan have less cross-linking density under irradiation and therefore possess smaller gel fractions. It has been reported that PVA hydrogels made by freeze-thawing are cross-linked physically with polymer crystallites acting as junction points (Kanaya, Ohkura, Kaji, Furusaka, & Misawa, 1994; Ricciardi, Auriemma, De Rosa, & Laupretre, 2004; Ricciardi et al., 2003; Willcox et al., 1999). As the physical cross-linking of PVA molecules can be greatly constrained by the presence of additives (Park, Park, & Ruckenstein, 2001), the addition of ws-chitosan will result in a decrease in gel fraction for the hydrogels prepared by freeze-thawing.

3.3. Swelling behavior of the hydrogels

Swelling capacity of hydrogels can be affected by many factors, such as the cross-linking density, the hydratability of the materials, the ionic strength and pH value of the media, as well as the temperature of the environment. Fig. 3 shows the pH sensitivity of PVA hydrogels, with or without ws-chitosan, and made by various methods. PVA hydrogels containing ws-chitosan exhibit the largest swelling capacity at pH 3.7, and when pH is above 7 the degree of swelling changes little. Hydrogel prepared by irradiation has the largest water-absorbing capacity and pH sensitivity while that prepared by freeze-thawing possesses the lowest swelling capacity and pH sensitivity (Fig. 3A). It is worth noting that hydrogel made by irradiation followed by freeze-thawing shows a larger swelling capacity compared with that made by freeze-thawing followed by irradiation. Hydrogels with more ws-chitosan show a larger pH dependence and swelling capacity while pure PVA hydrogel does not show pH sensitivity and has the smallest swelling capacity (Fig. 3B). The larger swelling capacity can be due to the lower cross-linking density as revealed in Section 3.2, while the larger pH sensitivity is due to the presence of more amino-groups in the hydrogels. It is well known that the amino-groups of ws-chitosan can be protonated in acidic media. This protonation induces electrostatic repulsions between the polymer segments. Besides, the protonation of the amino-groups may lead to the dissociation of hydrogen bonds among the polymers and thus to a relaxation of the macromolecular chains (Khalid, Agnely, Yagoubi, Grossiord, & Couarraze, 2002). When hydrogels are in neutral or alkaline media, the swelling is strongly reduced due to the deprotonation of the amino-groups. The pH dependence of the extracted

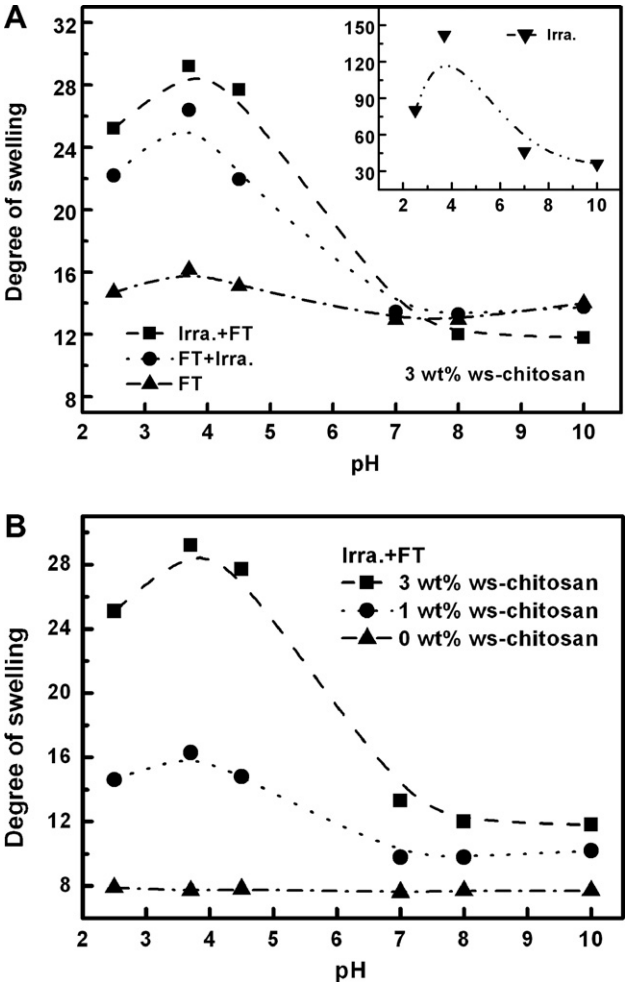

Fig. 3. Degree of swelling as a function of pH for (A) PVA/ws-chitosan hydrogels containing 3 wt% ws-chitosan and made by various methods, and (B) PVA/ws-chitosan hydrogels containing different amount of ws-chitosan made by irradiation followed by freeze-thawing. The concentrations of the buffer solutions are 0.01 mol/L and the content of PVA is 7 wt%.

PVA/ws-chitosan hydrogels is a strong indication that ws-chitosan was successfully introduced into the formed hydrogels, possibly via hydrogen bonds formed during freeze-thawing or by chemical cross-linking or grafting during irradiation.

The swelling kinetics of hydrogels made by various methods is shown in Fig. 4. More rapid swelling is observed for hydrogels prepared by irradiation than by freeze-thawing. To attain 70% of water in the hydrogel, 1 h is needed for the former whereas 2 h are required for the latter. An equilibrium state of swelling is achieved within 6 h for the hydrogel made by irradiation while more than 10 h is required for that prepared by freeze-thawing. The hydrogel prepared by irradiation followed by freeze-thawing shows similar behavior to that prepared by pure irradiation whereas the hydrogel prepared by freeze-thawing followed by irradiation behaves in a similar way to that prepared by pure freeze-thawing. It is found that ws-chitosan content does not influence the swelling kinetics much.

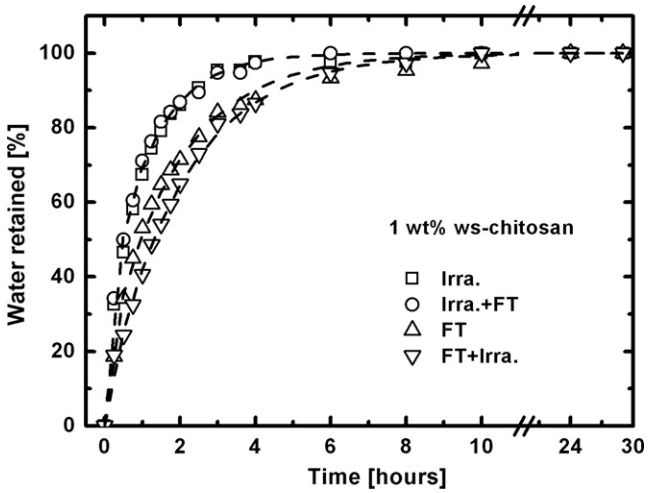

Fig. 4. Swelling kinetics of PVA/ws-chitosan hydrogels containing 7 wt% PVA and 1 wt% ws-chitosan prepared by various methods.

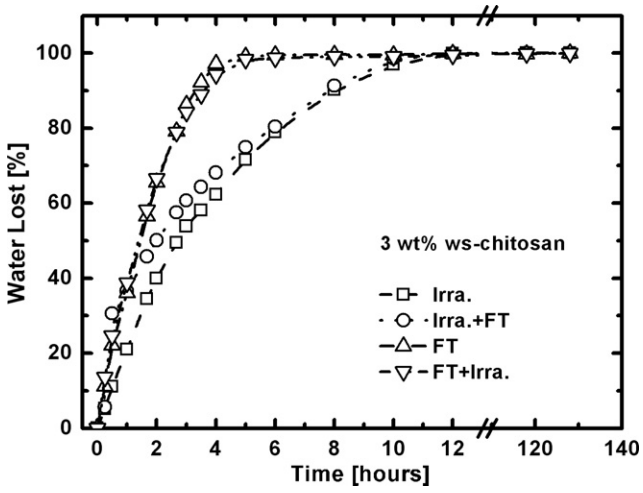

Fig. 5. Water evaporation from PVA/ws-chitosan hydrogels containing 7 wt% PVA and 3 wt% ws-chitosan and prepared by various methods.

Miranda, Lugao, Machado, & Ramanathan, (1999) reported that the swelling of cross-linked PVP occurred in three stages. In the first stage, the water absorption proceeds very fast with a rate depending on surface hydrophilicity and capillarity of small pores in the hydrogel. The second stage is an absorption stage governed mainly by diffusion mechanism. A constant of swelling is achieved after a sufficiently long time. In the last step, a minor and a very slow increase in water content continues based on a very slow network relaxation. In the present case, the experimental measurements shown in Fig. 4 were fitted with the following equation

$$S = \alpha(1 - e^{-t/\tau_1}) + \beta(1 - e^{-t/\tau_2})$$

where S was the percentage of water retained in the hydrogel, α and β were the relative contributions for the first and second swelling stages, respectively; τ_1 and τ_2 were the corresponding time constants of swelling. The third swelling stage was omitted in the analysis because of the limited testing time in our experiments. All the fittings (dashed lines in Fig. 4) were conducted by fixing τ_1 at 0.2 h (determined from the fitting data of hydrogels prepared by Irra. and Irra. + FT). It is found that the first stage takes a fraction of about 30% whereas the second stage (with a time constant of about 1.15 h) takes a fraction of about 70% for hydrogels prepared by irradiation and irradiation followed by freeze-thawing. For the hydrogels prepared by the other two methods, the time constant of the second stage is around 2 h and it indicates a much slower process. The fraction ratios of the first to the second stage differ for pure freeze-thawing and freeze-thawing followed by irradiation. It is around 17/83 for the former and 5/95 for the latter.

3.4. Water evaporation rate from hydrogel

The water evaporation from hydrogels in air at 37 °C and 40% humidity was investigated to examine the possibility of the hydrogels serving as wound dressing. As shown in Fig. 5, the water evaporation from hydrogels prepared by freeze-thawing alone and by freeze-thawing followed by irradiation proceeds rapidly compared to those prepared by the other two methods. The water evaporation is very fast in the initial 1 h and then slows down until it levels off in 4 h for hydrogels made by pure freeze-thawing and freeze-thawing followed by irradiation. For hydrogels made by the other two methods, the water evaporation rate is smaller and it takes 10 h to lose most of their water.

It is known that larger swelling capacity can prevent the accumulation of wound exudates, and a smaller evaporation rate can avoid changing the wound dressing too often. Therefore, hydrogels prepared by irradiation and irradiation followed by freeze-thawing are more suitable for use as wound dressing (Figs. 3 and 5).

3.5. Morphological studies

Cross-section morphologies of freeze-dried hydrogels containing 7 wt% PVA and 3 wt% ws-chitosan, and prepared by various methods are compared in Fig. 6. All the hydrogels exhibit porous structure. Hydrogels made by irradiation and irradiation followed by freeze-thawing show similar morphology with smaller pores while those made by freeze-thawing and freeze-thawing followed by irradiation have almost the same morphology with larger pores. It indicates that the microstructure of hydrogels is almost determined entirely by the first step of the processing. The structures give a better explanation to the observations of swelling and water evaporation behaviors of the hydrogels shown in Sections 3.3 and 3.4. Smaller pores of hydrogels prepared by irradiation and irradiation followed by freeze-thawing enable an effective capillary attraction (Fig. 4) and a slower evaporation of water (Fig. 5). The larger swelling capacity of hydrogels prepared by irradiation and irradiation followed by freeze-thawing is understandable as smaller pore size does not mean a smaller porosity.

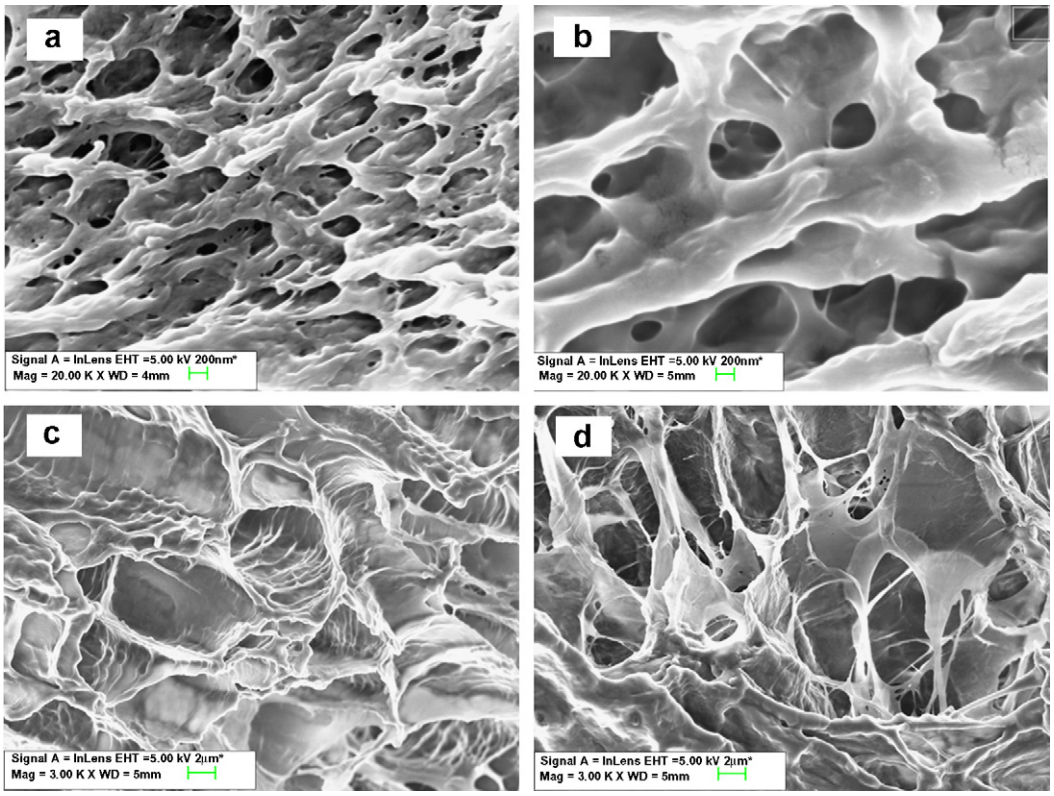

Fig. 6. SEM micrographs of the cross-section of hydrogels containing 7 wt% PVA and 3 wt% ws-chitosan and made by various methods. (a, b: hydrogels made by irradiation and irradiation followed by freeze-thawing, respectively, magnified 20,000 times; c, d: hydrogels made by freeze-thawing and freeze-thawing followed by irradiation, respectively, magnified 3000 times.)

3.6. Rheological behavior

Storage modulus (G') is a good measure of material stiffness (Ricciardi et al., 2003). It reflects the strength of hydrogels, i.e. the larger the G' the larger of the strength (Haines et al., 2005; Nugent et al., 2005; Sosnik & Sefton, 2005). Fig. 7 shows that the addition of 1 wt% ws-chitosan does not reduce the storage modulus much when compared with pure PVA hydrogels. However, G' decreases significantly when the content of ws-chitosan increases to 3 wt%. This can be explained by the fact that hydrogels containing more ws-chitosan have a smaller cross-linking density. Hydrogel made by irradiation have the smallest storage modulus (Fig. 7). It is too weak to be used as wound dressing although it possesses the advantage of transparency, large swelling capacity and suitable water evaporation rate as shown in Sections 3.1 and 3.3–3.4. It has been known that the freeze-thawing process can form PVA hydrogels with high elasticity and good mechanical strength. It is exciting to find that hydrogels prepared by irradiation followed by freeze-thawing have an even larger mechanical strength compared to those prepared by pure freeze-thawing and freeze-thawing followed by irradiation. Further investigations are ongoing to probe this phenomenon.

Fig. 8 shows the temperature dependence of the storage modulus for hydrogels made by various methods. It can be

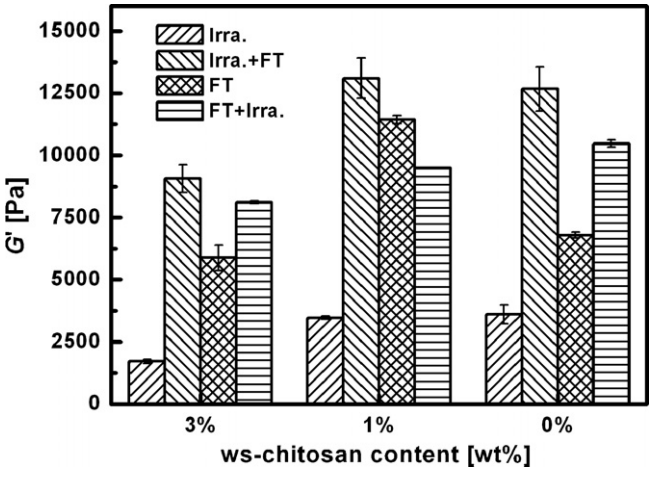

Fig. 7. Storage modulus at 1 rad/s for PVA/ws-chitosan hydrogels containing different amount of ws-chitosan and made by various methods. The content of PVA is 7 wt%.

seen that G' for hydrogels made by irradiation alone exhibits almost no temperature dependence due to the stable chemical cross-linking structure. The storage modulus of hydrogels made by pure freeze-thawing reduces significantly when the temperature is going above 50 °C due to the breakdown of the physical networks. The thermal stability of hydrogels made by combined irradiation and freeze-thawing, especially for hydrogels made by irradia-

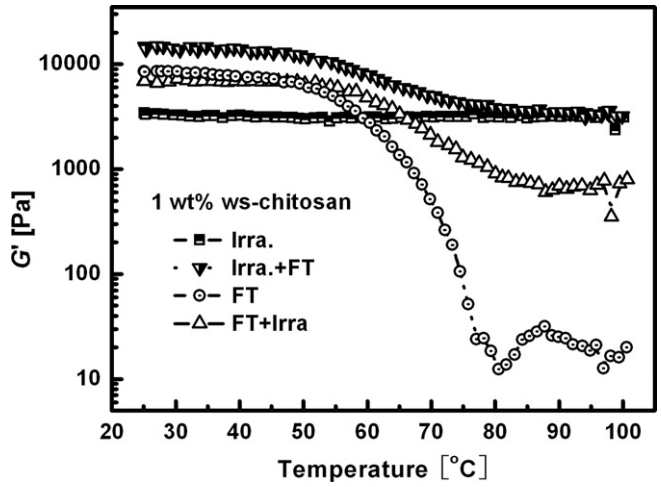

Fig. 8. Storage modulus at 1 rad/s as a function of temperature for hydrogels containing 7 wt% PVA and 1 wt% ws-chitosan, and made by various methods.

tion followed by freeze-thawing, is greatly improved compared to that made by pure freeze-thawing. A more than three time increase of storage modulus was achieved compared to that obtained by pure irradiation at temperatures below 50 °C. At temperatures above 50 °C, the storage modulus of hydrogels made by irradiation followed by freeze-thawing decreases with increase of temperature and comes close to the value achieved by pure irradiation, which is still much higher than that achieved by pure freeze-thawing and freeze-thawing followed by irradiation.

3.7. Antibacterial assessment

The antibacterial activity is a crucial parameter for wound dressing. Herein, the activity of PVA hydrogels, with or without ws-chitosan, against *E. coli* was checked. The results showed that *E. coli* only grew on media plates of the control and the PVA hydrogel without ws-chitosan. All the hydrogels with ws-chitosan could effectively constrain the growth of *E. coli* even when the content of ws-chitosan was 1 wt%. The antibacterial behavior of hydrogels containing ws-chitosan is due to their capability to bind the negatively charged bacteria to the positively charged amino-groups of ws-chitosan in the hydrogels (Tsai & Su, 1999).

4. Conclusions

A series of PVA hydrogels with or without ws-chitosan were synthesized by combined irradiation and freeze-thawing and their properties were investigated in comparison with those prepared by irradiation and by freeze-thawing alone. It is found that the microstructure and certain physical properties of hydrogels made by combined irradiation and freeze-thawing are mainly determined by the first processing step. Hydrogels made by irradiation alone have large swelling capacity, are transparent, and possess a suitable water evaporation rate. However, they are too weak to be used as wound dressing. Those made by freeze-thawing and freeze-thawing followed by irradiation have a high mechanical strength, but they are opaque in appearance, limited in water uptake, have too fast water evaporation rate and are not stable at high temperature. It is exciting to find that hydrogels made by irradiation followed by freeze-thawing have large swelling capacity, good thermal stability, larger mechanical strength, and possess adequate water evaporation rate and translucent appearance. With increasing ws-chitosan content, the gel fraction and the storage modulus decrease whereas the swelling capacity increases. All the hydrogels containing ws-chitosan show pH sensitivity and antibacterial activity against *E. coli* as a result of the presence of ws-chitosan.

Acknowledgement

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