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Evaluation of glucan/poly(vinyl alcohol) blend wound dressing using rat models

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Abstract

Aqueous mixture of β-glucan and poly(vinyl alcohol) (PVA) was cast into films and dried at 110 °C without chemical crosslinking. The content of glucan in the film varied from 7% to 50%. The hydrophilicity of the resulting films was evaluated with swelling tests, wet area diffusion tests, and water vapor transmission tests. The swelling ratio, the wetting ratio, and the water vapor transmission rate increased with the glucan content. When contacting water, glucan was released, and the percent release of glucan increased with the glucan content. The addition of glucan made the film more ductile than pure PVA. The results of hemocompatibility test showed no significant effect on the activated partial thromboplastin time (APTT) and thrombin time (TT) and minor adsorption of human serum albumin (HSA). On observing the wound healing of rat skin, the healing time was shortened by 48% using PVA/glucan film comparing to cotton gauze. Therefore, a wound dressing made of PVA/glucan can greatly accelerate the healing without causing irritation.

Keywords: Glucan; Poly(vinyl alcohol); Films; Wound dressing

1. Introduction

In order to overcome the poor biological performance of synthetic polymers and to enhance the mechanical characteristics of biopolymers, a new class of specifically designed materials, bioartificial polymeric materials has been introduced (Lazzeri, 1996; Cascone et al., 2001). These materials based on blends of both synthetic and natural polymers could be usefully employed as biomaterials or as low-environmental impact materials (Cascone et al., 1995, 1999; Lin et al., 2006; Lee et al., 2005; Vidyalakshmi et al., 2004; Sarti and Scandola, 1995). Many commercially available synthetic polymers, such as polyvinyl alcohol (PVA), show physicochemical and mechanical properties comparable to those biological tissues to be substituted (DeMerlis and Schoneker, 2003; Brinkman et al., 1991; Hassan et al., 2000; Li et al., 1998, 2004; Shaheen and Yamaura, 2003; Seabra and De Oliveira, 2004; Morita et al., 2000; Barbani et al., 2005). On the other hand, many biological polymers possess good biocompatibility but inadequate mechanical properties (Sudhamania et al., 2003). Improvements in the characteristics of synthetic biomaterials could be achieved by the addition of biological macromolecules such as fibrin, collagen, elastin and glycosaminoglycans. The resulting materials can exhibit appropriate mechanical properties as well as biocompatibility (Cascone et al., 1995).

(1-3),(1-6)-β-Glucan is a water-soluble and biodegradable polymer fermented from plant incubation. (1-3),(1-6)-β-Glucan can boost the immune response by activating macrophage cells. It consists of β-(1-3) linked-D-glucose residues with one β-(1-6) linked D-glucosyl group for every three glucose residues. β-Glucan is effective against allogeneic, syngeneic and autochthonous tumors. It shows antibacterial and antiviral effects and exhibits the wound healing activity (Lee et al., 2003; Bohn and BeMiller, 1995). In the last few years the attention has been focused on the study of bioartificial materials based on polysaccharides. Polysaccharides are biological polymers that can be obtained from different sources: microbial sources such as dextran and gellan, animal sources such as chitosan, and vegetal sources such as starch. Several advantages can derive from the use of these macromolecules. However, studies on glucan in biomedical applications are scarce. The only one was using sponges of glucan and gelatin for artificial skin (Lee et al., 2003).

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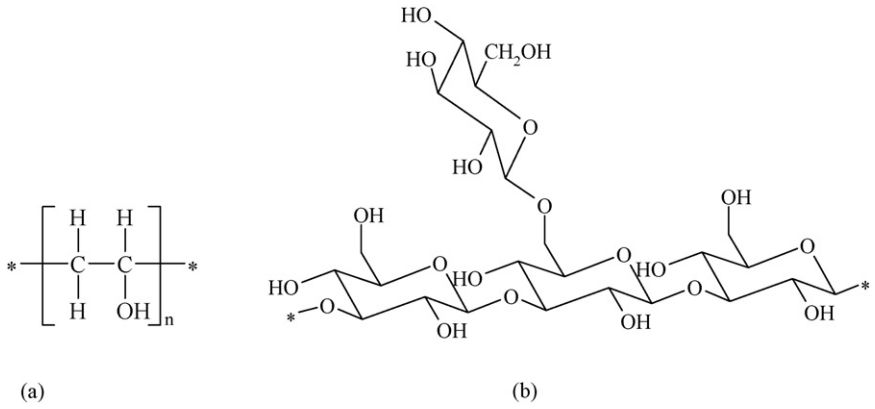

Fig. 1. Chemical structures of (a) polyvinyl alcohol and (b) (1-3),(1-6)-β-glucan.

Polyvinyl alcohol is often used in biomedical applications. It is a water-soluble synthetic polymer with excellent film forming, emulsifying, and adhesive properties. This polymer has outstanding resistance to oil, grease, and solvents. Addition of PVA to an elastomer material like gellan has a marked influence on its properties depending on the modification achieved, its ratio and the compatibility of the individual polymers. There are many studies on the compatibility of blends of polymeric materials, but there are very few on the preparation and characterization of biodegradable polymer blends (Lee et al., 2003). For instance, PVA/PVP/chitosan hydrogels were studied for wound dressing (Yu et al., 2006). Although a hydrogel wound dressing can provide a wet environment for the wound, it would also provide an environment for the propagation of bacteria on the wound surface. Thus chitosan was employed to endow a hydrogel wound dressing with antibacterial ability. Although chitosan is not cytotoxic, chitosan seems to be cytostatic toward fibroblasts, that is, inhibiting cell proliferation (Chatelet et al., 2001). The poor hydrophilicity and cytocompatibility hinder the usage of chitosan in the field of tissue engineering.

The aim of this work is to study the wound dressing applicability of glucan-blending PVA films prepared via casting method. There are two advantages using this blend as wound dressing. Glucan would be released from the blend film during the healing process. Glucan is reported to be promoting cell proliferation (Lee et al., 2003). Thus releasing glucan would be able to accelerate the healing of wound. Furthermore, the PVA can provide the mechanical strength of wound dressing, thus the PVA/glucan film would be more suitable for wound healing applications than in hydrogel form. In this work, these PVA/glucan films were subject to hydrophilic tests, tensile tests, protein adsorption, cytocompatibility, and blood coagulation tests. Animal tests including irritation tests and wound healing experiments were also performed. The results of this work would demonstrate the applicability of PVA/glucan film as a wound dressing.

2. Experimental

2.1. Materials

Polyvinyl alcohol was purchased from Chang Chun Petrochemical Co. Ltd. (Taiwan) of which the hydrolysis degree was 98.5–99.2% with a molecular weight of about 72 kDa, and was used without further purification. β-Glucan stock (11 wt.%) was purchased from Geneferm Bio Technology Co. Ltd. (Taiwan) which was from mushroom strain fermentation with a wide distribution molecular weight of about 2 MDa. Fig. 1 shows the molecular structures of PVA and β-glucan.

2.2. Preparation of blend films

Firstly, PVA powder was dissolved in deionized (DI) water at 90 °C for 2 h under stirring to form a homogeneous solution of 16 wt.%. The solution was cooled to room temperature. Then a volume of glucan stock solution was mixed with the PVA solution at seven ratios, as listed in Table 1. When the volume of glucan solution was higher than 50%, the mixture was too dilute for casting film. Each mixture was mixed and then centrifuged for 5 min at 3000 rpm. Without any chemical treatment, the mixture was cast on a Mylar sheet to a uniform dry

Table 1
The preparation and the compositions of PVA/glucan blends

Ingredient	Designation						
	PVA	G10V90	G20V80	G30V70	G40V60	G50V50	G60V40
Glucan (11 wt.%) (ml)	0	10	20	30	40	50	60
PVA (16 wt.%) (ml)	100	90	80	70	60	50	40
Glucan content (wt.%)	0	7.1	14.7	22.7	31.4	40.7	50.8

thickness of about 30 μm by a roller coater (Autocoater, MTS, Germany). The drying temperature of the autocoater was set at 110 °C and the drying time was set at 3 min. The resulting films were preserved in a desiccator.

2.3. Hydrophilicity evaluation

Because the hydrophilicity of PVA/glucan films was too high to be distinguished by contact angle measurements, other methods were employed to evaluate the hydrophilicity. In the water diffusion area test, an aliquot of water of 20 μl was dropped on the film. The wetting area (i.e. the water diffusion area) expanded with the time and was monitored. The wetting ratio can then be calculated as follows:

$$\text{Wetting ratio} = \frac{\text{water diffusion area at } t}{\text{initial water diffusion area}}$$

To measure the swelling behavior of the films, samples of 1 cm × 1 cm were dried in an oven for 2 h at 105 °C. Afterwards, the samples were placed in a humidifying chamber of 90% relative humidity at 37 °C, and weighed at specific time points. The swelling ratios of the samples were calculated as follows:

$$\text{Swelling ratio} = \frac{W_{\text{wet}}}{W_{\text{dry}}}$$

where W_{wet} and W_{dry} represent the weights of the film in the wet and dry states, respectively.

The water vapor transmission tests were conducted according to JIS 1099A standard method. A circular piece of the specimen was fastened over the top of a cup of 7 cm in diameter containing 50 g of $CaCl_2$. The cup was then placed in an incubator of 90 ± 5% RH at 40 ± 2 °C. The water vapor transmission rate (WVTR) was calculated as follows:

$$\text{WVTR} = \frac{W_2 - W_1}{S} \times 24\,(\text{g/m}^2\text{-day})$$

where W_1 and W_2 are the weights of the whole cup at the first and second hours, respectively, and S is the transmitting area of the specimen.

2.4. In vitro release of glucan

The *in vitro* release tests were carried by soaking pieces of moisture-swollen samples (2 cm × 2 cm) in 20 ml/piece of physiological saline at 25 °C. At specified time intervals, both the wet and dry weights of the sample were measured. The release percent of glucan was calculated as follows:

$$\text{Release } \% = \frac{W_{G0} - W_{Gt}}{W_{G0}} \times 100\%$$

where W_{G0} and W_{Gt} are the initial weight of glucan and the residual weight of glucan at time t, respectively.

2.5. Tensile tests

The tensile strength and breaking elongation of the blend films were measured with a tensile tester (MTS 810, Material Test System, USA) according to ASTM D882-02. The dry samples were prepared by vacuum drying at 40 °C over night. The specimens were cut into a specific dog-bone shape (11.5 cm long, 2.5 cm wide at the ends and 0.6 cm wide in the middle). The thickness of each specimen was measured. The measurement was conducted at a cross-head speed of 10 mm/min under a pre-load of 10 kg.

2.6. Adsorption of proteins

A piece of blend film of 1 cm × 1 cm was immersed in 5 ml of pH 7.4 PBS containing 2 mg/dl human serum albumin (HSA) (M_W 66 kDa, Calbiochem, USA) at 37 °C for 24 h under 100 rpm shaking. Afterwards, the samples were gently taken out and rinsed five times with PBS, followed by placing in 1 wt.% aqueous solution of sodium dodecyl sulfate (SDS) and shaken for 60 min at room temperature to remove the protein adsorbed on the surface. The HSA content of each sample was measured using the BCA reagents (Pierce). The absorbance at 562 nm was measured using a spectrometer (UV-1200, Shimadzu, Kyoto, Japan) (Lin et al., 2006; Liu et al., 2005).

2.7. Blood coagulation test

The *in vitro* coagulation times, including activated partial thrombin time (APTT) and thrombin time (TT) were measured using a blood coagulation tester (CA-50, Sysmex Corp, Japan). A sample of 1 cm × 1 cm was soaked in 500 μl platelet poor plasma (PPP) and incubated at 37 °C for 30 min. Afterwards, 50 μl of the plasma was pipetted to the tester, stood for 1 min, followed by adding 50 μl APTT reagent. After incubating for 3 min, 50 μl of $CaCl_2$ were added. The APTT was then determined. To measure TT, 100 μl of the plasma was pipetted to the tester, stood for 2 min, followed by adding 50 μl TT agent. The reading of TT was then obtained.

2.8. Determination of cytotoxicity

The *in vitro* cytotoxicity of blend films was measured according to ASTM F813. After culturing for 1, 2, 3, 4 and 7 days, the viability of fibroblasts was determined by MTT assay. The MTT reagent is a pale yellow substrate which produces a dark formazan product when incubation with viable cells. The level of the reduction of MTT into formazan can reflect the level of cell metabolism. The method of Mosmann (1983) was modified and used in this study. At each period of culturing time, 100 μl of MTT (Sigma, USA) solution was added to each well. After 3 h incubation at 37 °C, dimethyl sulfoxide (DMSO) of 200 μl was added to dissolve the formazan crystals. The dissolvable solution was jogged homogeneously about 15 min by the shaker. The optical density of the formazan solution was read on an ELISA plate reader (ELx 800, Bio-tek, USA) at 540 nm. All experiments were repeated three times, and the results are expressed as mean standard deviation. The differences were evaluated by using Student's t-test. Significance was assessed at the $p < 0.05$ level of confidence.

2.9. Skin irritation test

The primary skin irritation test was performed according to the ISO10993-10 using three healthy male Guinea pigs. The back of the animals was clipped free of fur and divided into four sites 24 h before applying the samples. The material (2.5 cm × 2.5 cm) was applied to only two sites, while Rayon non-woven sample was applied to the other two sites as the controls. All the sites were covered by gauze and the back of the animal was wrapped with a non-occlusive bandage. After 4 h, the bandage and the test materials were removed. After 1 h, these four sites were examined for skin irritation and the observation was repeated after 24, 48, and 72 h. The score of primary irritation (SPI) was calculated according to ISO10993-10 for each animal and the primary irritation index (PII) was calculated as the arithmetical mean of SPI values (Mazzanti et al., 2005).

2.10. Wound healing test

Male Wistar rats, aged 3 months and weighing 450–550 g, were obtained from the National Science Council of ROC. Prior to the test, the rats were anaesthetized with pentobarbital. After removing the dorsal hair of the rats with an electric razor, 10% aqueous betadine and 70% alcohol were employed to sterilize the dorsal area of the animals. Then four full thickness wounds with a surface area of 2 cm × 2 cm were created from the back. Each wound was covered with an equal size of the specimen, or cotton gauze, or the commercial product (Nexcare™, 3M, USA) for comparison. On top of the wound dressings, a piece of Tegaderm™ (3M, USA) was applied. Treated rats were placed in individual cages and the healing wounds were observed on the 1st, 4th, 7th, 11th, 14th and 21st days using a digital camera. The degree of healing was expressed as the wound contraction ratio (WCR):

$$\mathrm{WCR} = \frac{A_0 - A_t}{A_0} \times 100\%$$

where A_0 and A_t are respectively the initial area and the wound area at time t. Wound tissue was dissected, fixed with 10% phosphate-buffered formalin, and stained with hematoxylin and eosin (HE) reagents for histological observations (Mi et al., 2001).

3. Results and discussion

3.1. Hydrophilicity of the blend films

Table 2 lists the results of the hydrophilicity tests including swelling ratio, wetting ratio, and water vapor transmission. All these are important for evaluating wound dressing materials. The addition of glucan caused the swelling ratio to increase from 1.18 (PVA) to 1.51 (G60V40). The wetting ratio also increased with the glucan content from 2.00 (PVA) to 4.57 (G60V40). Furthermore, the WVTR increased with the glucan content from 1103 (PVA) to 3310 (G60V40). All these results indicate that the addition of glucan can improve greatly the hydrophilicity of PVA, thus the blend film might be suitable for wound dressing. Similar results were reported for PVA/chitosan blend films (Vidyalakshmi et al., 2004). The WVTR values increased with the increase of chitosan content, because chitosan was more hygroscopic than PVA.

Table 2
Swelling ratios, wetting ratios, and water vapor transmission rates of the PVA/glucan films

Sample	Swelling ratio	Wetting ratio	WVTR (g/m^2-day)
PVA	1.18 ± 0.13	2.00 ± 0.11	1103 ± 24
G10V90	1.22 ± 0.05	2.34 ± 0.20	1188 ± 19
G20V80	1.27 ± 0.03	2.60 ± 0.10	1273 ± 21
G30V70	1.30 ± 0.10	2.80 ± 0.10	1698 ± 42
G40V60	1.35 ± 0.04	3.23 ± 0.19	2292 ± 33
G50V50	1.42 ± 0.02	3.83 ± 0.09	3226 ± 25
G60V40	1.51 ± 0.04	4.57 ± 0.15	3310 ± 56

An ideal wound dressing must control the water loss from a wound at an optimal rate. Lamke et al. (1977) reported the evaporative water loss for normal skin as 204 ± 12 g/m^2-day and that for injured skin can range from 279 ± 26 g/m^2-day for a first degree burn to 5138 ± 202 g/m^2-day for a granulating wound. The water vapor transmission of a wound dressing should prevent excessive dehydration as well as build up of exudates. It has been recommended that a rate of 2000–2500 g/m^2-day would provide adequate level of moisture without risking wound dehydration (Queen et al., 1987). High WVTR would lead to total dehydration of the wound surface, causing the adhering of the dressing to the wound. Those values of WVTR in Table 2 were close to the range appropriate for maintaining a proper fluid balance on the wound, which can facilitate cellular migration and enhance reepithelialization.

3.2. Tensile properties of the PVA/glucan blend films

Figs. 2 and 3 show the tensile properties of PVA/glucan films. The Young's modulus of the blend film decreased with

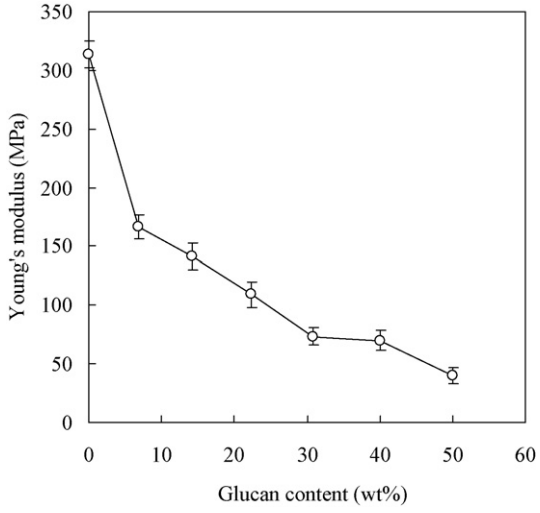

Fig. 2. Young's modulus of PVA and PVA/glucan blend films.

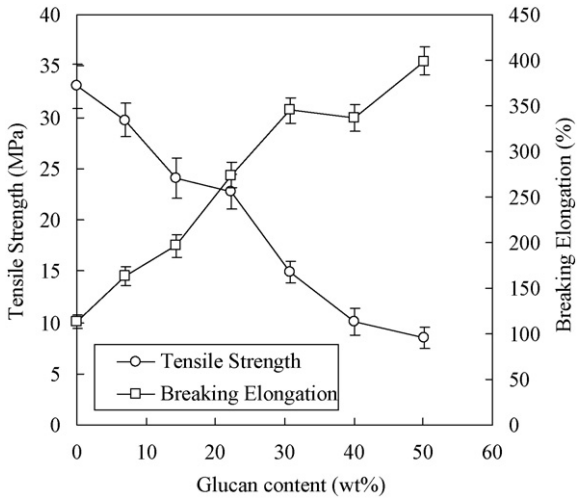

Fig. 3. Tensile strength and breaking elongation of PVA and PVA/glucan blend films.

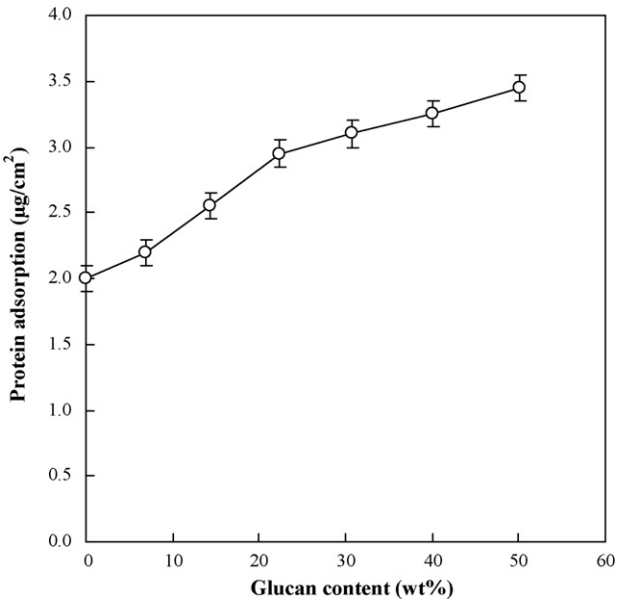

Fig. 4. The dependence of the adsorption amount of HSA on the glucan content.

the increase of glucan content from 314 MPa (PVA) to 40 MPa (G60V40), as shown in Fig. 2. This suggests that the blending of glucan made the material less stiff. In addition, Fig. 3 shows that with the increase of glucan content, the tensile strength decreased from 33 MPa (PVA) to 8.5 MPa (G60V40) while the breaking elongation increased from 113% (PVA) to 399% (G60V40). These results indicate that the blending of glucan caused the sample to become more ductile. This can be attributed to the hydrogen bonding or intermolecular interactions. Glucan is a polysaccharide with side groups. When blending with linear PVA, the orderliness of the molecular arrangement was interfered by glucan, thus the mechanical strength was reduced. The resulting blend became a flexible and elastic material.

3.3. Adsorption of serum proteins onto matrix surfaces

When blood contacts a foreign material, such as a hemodialyzer, catheter or angioaccess, plasma proteins are always adsorbed onto the material surfaces, and provoke the adhesion of platelets, white blood cells and some red blood cells onto the plasma protein layer.

Fig. 4 shows that the adsorption of human serum albumin (HSA) increased with the increase of glucan content. The adsorption of protein onto substrates depends significantly on the surface characteristics, such as hydrophilicity, roughness, charge, or chemistry. The amount of HSA adsorbed onto the PVA/glucan surface was about 1.1–1.7 times higher than that of PVA. Because both PVA and glucan carry no charge, the adsorption of serum proteins can be attributed to the increase of hydrogen bonds. Glucan is a polysaccharide with three hydroxyl groups and two ether groups per six carbons, thus it provides more hydrogen bonding sites than PVA which has only one hydroxyl groups per two carbons, as shown in Fig. 1. Therefore the adsorption of HSA was improved by blending glucan with PVA.

3.4. Blood compatibility

The coagulation activity was evaluated based upon the coagulation time including activated partial thromboplastin time (APTT), and thrombin time (TT). Table 3 shows that the coagulation time changed little with the blending of glucan. The APTT varied around 34 s while TT varied around 14 s. This indicates that PVA/glucan blend would not activate the blood coagulation. Gauze and Nexcare™ had similar coagulation times. Therefore the blood compatibility of PVA/glucan film as a wound dressing was comparable to those of gauze and Nexcare™.

3.5. In vitro cytocompatibility of the films

The cytotoxicity of the matrices is very important for their tissue engineering applications. The cytotoxicity of PVA/glucan was evaluated based on the MTT assay. Fig. 5 shows that fibroblasts proliferated well on the G40V60 blend film surface in seven culture days. No significant difference was observed between the control (tissue culture polystyrene dish) and the G40V60 film. The spectrophotometer readings indicated that the fibroblasts seeded onto G40V60 film were able to convert the MTT into a

Table 3
The APTT and TT of the PVA/glucan films, gauze, and Nexcare™

Sample	APTT (s)	TT (s)
Blank	34.2 ± 0.6	16.9 ± 1.2
PVA	32.9 ± 0.5	15.6 ± 1.6
G10V90	34.3 ± 0.7	16.3 ± 1.6
G20V80	34.8 ± 1.0	16.3 ± 0.4
G30V70	34.5 ± 1.5	12.8 ± 1.0
G40V60	28.8 ± 1.5	12.8 ± 0.2
G50V50	32.0 ± 0.7	13.0 ± 1.1
G60V40	33.8 ± 1.6	13.4 ± 0.7
Gauze	33.6 ± 1.0	16.3 ± 0.8
Nexcare™	33.9 ± 1.1	17.4 ± 1.1

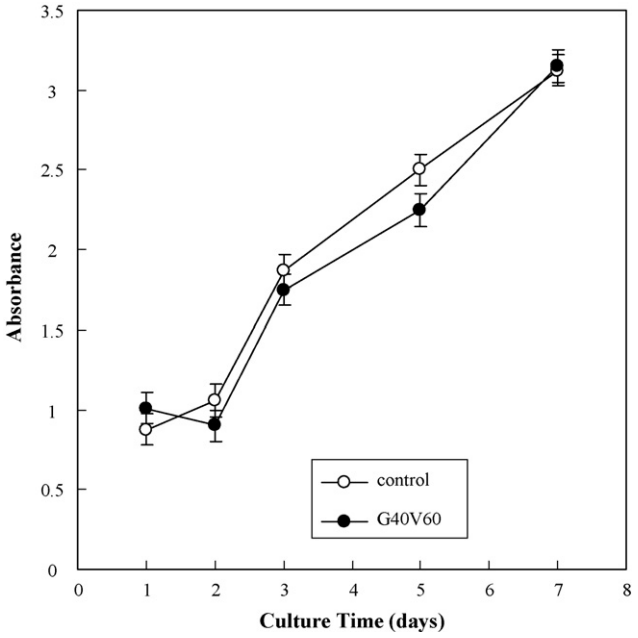

Fig. 5. The proliferation of fibroblast cultured on control (TCPS) and G40V60 film.

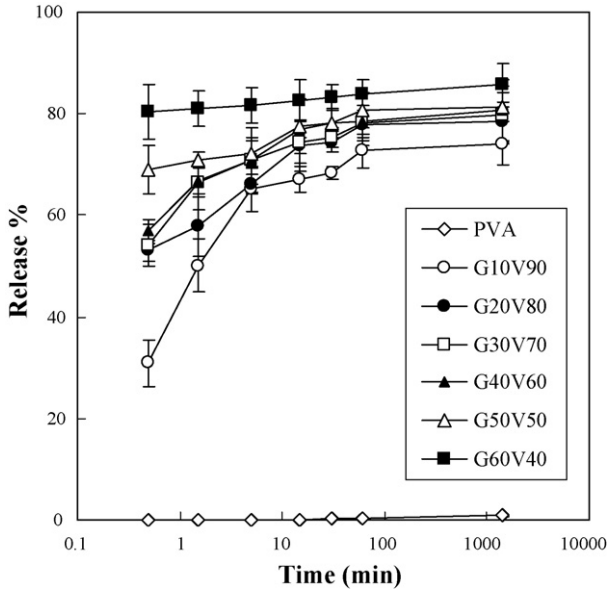

Fig. 6. The cumulative release of glucan from PVA and PVA/glucan blend films with respect to time.

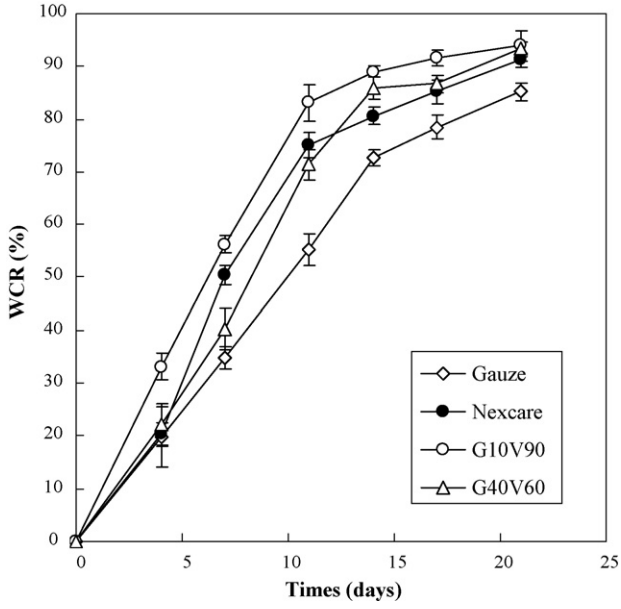

Fig. 7. Wound contraction ratios of PVA and PVA/glucan blend films at different times.

blue formazan product. The tendency of these two curves was similar. This suggests that the novel PVA/glucan film should be noncytotoxic. Therefore, it may be a candidate for biomedical applications.

3.6. In vitro release studies

In analyzing the results of glucan dissolution, the glucan loss was determined as the weight percent of the films. Therefore, the results were normalized by the weight of each original film containing both glucan and PVA. All films had similar initial weights before being placed in saline solution. PVA/glucan films were exposed to saline solution during a 24 h time period for observing the rate of release.

Fig. 6 shows the cumulative release of glucan from the samples with respect to time. The percent release of the blend film increase with the increase of glucan content from 30% (G10V90) to 80.5% (G60V40) at 0.5 min. After 90 min, the percent release of glucan was almost leveled. This suggests that the blending of

Table 4
Score of erythema and edema after application of test material

Animal	Day 1				Day 2				Day 3				SPI	PII
	1	2	3	4	1	2	3	4	1	2	3	4		
A														
Erythema	0	1	0	1	0	0	0	0	0	0	0	0	2/12 − 0/12 = 0.167	
Edema	0	0	0	0	0	0	0	0	0	0	0	0		
B														
Erythema	0	0	0	0	0	0	0	0	0	0	0	0	0/12 − 0/12 = 0	(0.167 + 0 + 0.083)/3 = 0.083
Edema	0	0	0	0	0	0	0	0	0	0	0	0		
C														
Erythema	0	0	0	1	0	0	0	0	0	0	0	0	1/12 − 0/12 = 0.083	
Edema	0	0	0	0	0	0	0	0	0	0	0	0		

glucan can facilitate the release of glucan. This can be attributed to the swelling of the matrix. The swelling ratio increased with the glucan content, thus glucan can be more easily released.

3.7. Test for irritation

The irritation test of the matrix is very important for the attachment to the skin. Table 4 lists the results of the test. The SPI of the animals A and C are less than 0.2, while that of animal B was 0. The PII of the test material was 0.083, which was less than 0.4. Thus according to ISO10993-10:1996, the primary skin irritation of the PVA/glucan impression material tested can be considered negligible.

3.8. Wound healing performance

Fig. 7 shows the WCR of the wounds treated with different means. By observing the wound area at definite intervals of time, the reduction in wound defect area was calculated. In case of G10V90-treated mice, healing was rapid and about 90% wound closure was achieved within 14 days. More precisely, substantial wound contractions in the G10V90-treated wounds were observed and more than 50% wound closure was achieved in the first 7 days. Since glucan can accelerate wound healing (Lee et al., 2003), the acceleration of wound healing in this experiment can be attributed to the release of glucan from PVA/glucan films, as mentioned in Section 3.5. Therefore, the results demonstrate that blending proper amount of glucan with PVA can accelerate the healing process. In the literature, glucan was speculated to reduce the time for fibroblasts to invade wound tissue and by early synthesis of new skin tissue (Yamada et al., 1999; Lee et al., 2003).

The healing of G40V60-treated wound was slower than G10V90-treated wound. Only after 14 days the WCR of G40V60-treated wound surpassed that of the Nexcare™. After 21 days, the WCR of G40V60-treated wound reached 93% and that of the Nexcare™ reached 91%. On the other hand, the control (gauze-treated wounds) healed slowly and about 85%

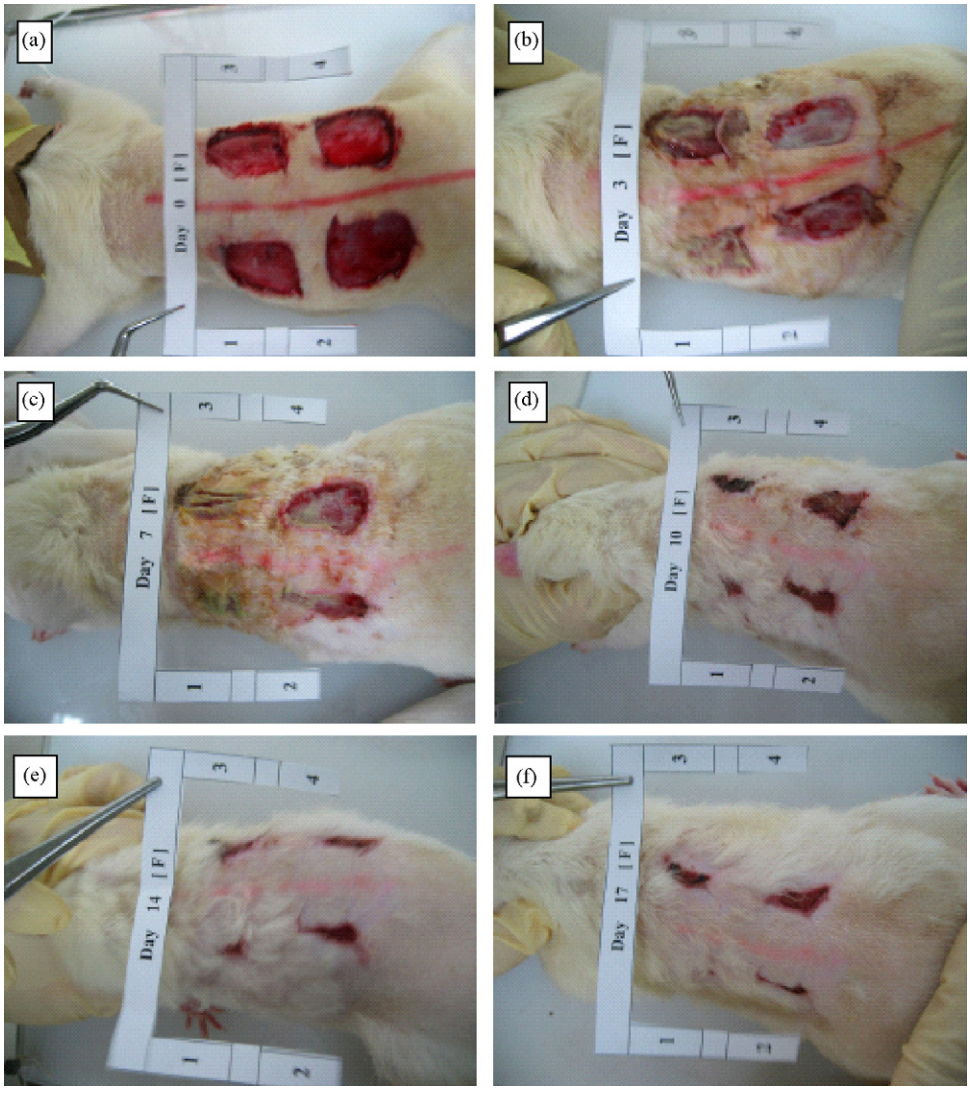

Fig. 8. Photographs of macroscopic appearance of wound repair covered with (1) G10V90, (2) G40V60, (3) Nexcare™, (4) cotton gauze at different time: (a) day 0, (b) day 3, (c) day 7, (d) day 10, (e) day 14, and (f) day 17.

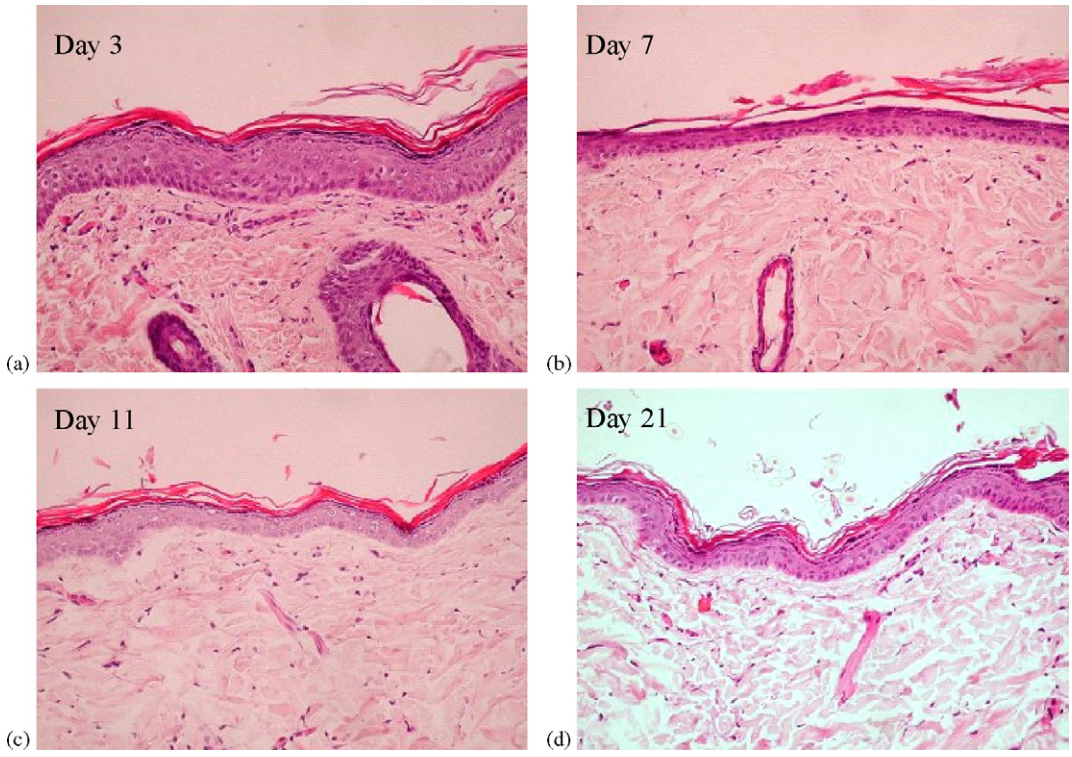

Fig. 9. Histological findings of wound: (a) day 3, (b) day 7, (c) day 11, and (d) day 21.

wound closure was achieved only after 21 days. This indicates that PVA/glucan films can be comparable to the Nexcare™ for wound healing. Furthermore, higher glucan content may slow down the healing process in the inflammatory phase. The amount of glucan in the G10V90-treated wound was about one-fifth of that in the G40V60-treated wound. Apparently the later was too high and might retard the healing in the early phase. Although glucan can accelerate the proliferation of fibroblast, it was shown to reduce the migration rate of fibroblast (Son et al., 2005). The later may be the reason that the healing was retarded by higher glucan content. Therefore, the blending of glucan must be in a proper ratio to accelerate wound healing, otherwise the effect may be reversed.

The wound healing efficacy of the film was evaluated in a full thickness wound model. Each wound (G10V90, G40V60, Nexcare™, and gauze) was observed after 3, 7, 10, 14 and 17 days of treatment. Fig. 8 shows that the wound shrinks with the time. After 14 days, G10V90 and G40V60 wounds appeared to be healed. After 17 days, the WCR was in the order of G10V90 > G40V60 > Nexcare™ > gauze. Although the WCR of Nexcare™ was higher than that of gauze after 10 days, hemorrhage on the skin was observed on the third day and scab was present on the 10th day. Winter reported that epithelialization is retarded by the dry scab and that epithelialization can be accelerated if the wound is kept moist (Winter, 1962). One explanation for this was that keratinocytes migrated more easily over a moist wound surface than underneath a scab (Winter and Scales, 1963). This is the reason why the WCR of G40V60 was higher than that of Nexcare™ on the 14th day, since no scab was observed for the wounds treated with either G10V90 or G40V60 films. In the case of gauze-treated wound, because gauze would adhere to the wound, the wound was damaged due to the replacement of new gauze.

3.9. Histological examination

Fig. 9 shows the healing pattern of G10V90-treated wound after 3, 7, 11 and 21 days of treatment. After 3 days, the wound was in the so-called inflammatory phase. Inflammatory phase is a normal and necessary prerequisite to healing (Kirsner and Eaglstein, 1993). In this phase, inflammatory granulation tissue and angiogenesis were observed in dermis. In addition, vasodilatation occurred that increased the permeability of blood vessels to deliver neutrophils and monocytes that differentiated into macrophages to phagocytize microbes. After 7 days, the wound evolved into the proliferative phase. The granulation tissues disappeared and were replaced by collagen fibers. The epidermis became thinner. Although capillary was still present in the dermis, vasodilatation reduced greatly. After 11 days, inflammatory cells, granulation tissues, and blood vessels were absent. Immature collagen fibers filled the dermis. After 21 days, the dermis was filled with fibroblasts and collagen fibers. In the epidermis, the epithelia were orderly arranged and a stratum corneum was observed.

4. Conclusions

Polyvinyl alcohol can be blended with glucan in aqueous solution and cast into films after drying at 110 °C for 3 min. Because glucan was not covalently bonded with PVA, it can be released to facilitate wound healing. Therefore these PVA/glucan films were applicable as wound dressing. Although

the tensile strength of PVA/glucan film was lower than those of native PVA films, the breaking elongation was significantly higher. With the increase of glucan content, the tensile strength of PVA film decreased whereas the breaking elongation of the film increased. The WVTR of PVA/glucan blend films was three times of that of native PVA. The percent release of glucan increased with the glucan content. These PVA/glucan films exhibited negligible irritation to skin. On observing the wound healing of rat skin, the wound contraction ratio can reach 83% after treating with PVA/glucan film for 11 days, while that was 85% when treating with cotton gauze for 21 days. Thus the healing time was significantly shortened by 48%. This accelerating effect can be attributed to the release of glucan. However, higher glucan concentration might reduce the cell mobility, thus the blending of glucan with PVA should be in an appropriate blending ratio to facilitate wound healing. The results of this work thus demonstrated that PVA/glucan films in a proper blending ratio could be used as a wound dressing that can accelerate wound healing.

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