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Gel characterisation and *in vivo* evaluation of minocycline-loaded wound dressing with enhanced wound healing using polyvinyl alcohol and chitosan

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ABSTRACT

The purpose of this study was to develop a minocycline-loaded wound dressing with an enhanced healing effect. The cross-linked hydrogel films were prepared with polyvinyl alcohol (PVA) and chitosan using the freeze-thawing method. Their gel properties, *in vitro* protein adsorption, release, *in vivo* wound healing effect and histopathology were then evaluated. Chitosan decreased the gel fraction, maximum strength and thermal stability of PVA hydrogel, while it increased the swelling ability, water vapour transmission rate, elasticity and porosity of PVA hydrogel. Incorporation of minocycline (0.25%) did not affect the gel properties, and chitosan hardly affected drug release and protein adsorption. Furthermore, the minocycline-loaded wound dressing composed of 5% PVA, 0.75% chitosan and 0.25% drug was more swellable, flexible and elastic than PVA alone because of relatively weak cross-linking interaction of chitosan with PVA. In wound healing test, this minocycline-loaded PVA-chitosan hydrogel showed faster healing of the wound made in rat dorsum than the conventional product or the control (sterile gauze) due to antifungal activity of chitosan. In particular, from the histological examination, the healing effect of minocycline-loaded hydrogel was greater than that of the drug-loaded hydrogel, indicating the potential healing effect of minocycline. Thus, the minocycline-loaded wound dressing composed of 5% PVA, 0.75% chitosan and 0.25% drug is a potential wound dressing with excellent forming and enhanced wound healing.

1. Introduction

The ideal wound dressing maintains a moist environment around the wound and absorbs the exudates from the wound surface (Turner, 1979). Acute and partial thickness wounds showed a significant increase in re-epithelialisation rates when they were maintained in a moist local environment (Hinnman and Maibach, 1963; Winter, 1962). Hydrogels, three-dimensional cross-linked hydrophilic polymers with a very high intrinsic content of water, can provide a moist environment to the wound area and absorb the exudates (Ajji et al., 2008).

Many hydrogels are prepared by physical methods such as repeated freezing and thawing, chemical methods using a covalent cross-linking agent including boric acid, glutaraldehyde and formaldehyde, or radiation methods using electron beam or γ-irradiation (Hassan et al., 2000; Yang et al., 2004). Polyvinyl alcohol (PVA) hydrogels prepared with a freeze-thawing method have been studied for biomedical and pharmaceutical applications because of their non-toxicity, non-carcinogenicity and good biocompatibility (Stauffer and Peppas, 1992).

In our previous reports, nitrofurazone-loaded PVA/sodium alginate hydrogels (Kim et al., 2008a) and clindamycin-loaded PVA/sodium alginate hydrogels (Kim et al., 2008b) could not improved the healing effect compared to conventional products. Thus, in this study, to develop an effective minocycline-loaded wound dressing with an enhanced healing effect, chitosan and minocycline were used instead of sodium alginate and other drugs. Chitosan is a linear copolymer of β (1-4)-linked 2-acetamido-2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β-D-glyco-pyranose. It is biodegradable, non-toxic and hemostasic (Ge et al., 2000). Specially, it gives antifungal activity, promotes normal tissue regeneration and recovers the original tensile strength of

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Table 1
Compositions of PVA/chitosan-based hydrogels.

Ingredient	I	II	III	IV	V	VI
PVA (g)	10	7.5	6.25	5	3.75	2.5
Chitosan (g)	–	0.375	0.563	0.75	0.938	1.125
Water (ml)	100	100	100	100	100	100

Each composition contained no drug or 0.25% (w/v) minocycline.

the wound by speeding the fibroblast synthesis of collagen (Chung et al., 1994). Minocycline was used in this study because it has been used topically in the treatment of superficial infections of the skin (Aoyagi et al., 2007; Gehrig and Warshaw, 2008). PVA is a semicrystalline copolymer of vinyl acetate and vinyl alcohol. It has been widely utilised in the chemical and medical industries because of its good biocompatibility, non-toxicity, hydrophilicity and fibre/film forming ability (Cascone et al., 1999). Furthermore, unlike other wound dressing reports (Aoyagi et al., 2007; Huang and Yang, 2008; Kim et al., 2008a,b), release test and histopathology test were carried out in order to evaluate a correct release mechanism and profound wound healing effect.

2. Materials and methods

2.1. Materials

PVA (typical average Mw = 146,000–186,000; +99% hydrolysed) and human serum albumin (HSA) (Mw = 66 kDa, albumin: 97.31%) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA) and Fluka Co. (Buchs, Switzerland), respectively. Chitosan (viscosity = 800–2000 cps; >75% deacetylation) and human plasma fibrinogen (HPF) (Mw = 341 kDa, clottable proteins >95%) were purchased from Calbiochem Co. (San Diego, CA, USA). Minocycline hydrochloride was supplied from SK Chemical Co. (Suwon, South Korea). Conventional wound dressing product (Medifoam™) was purchased from Ildong Pharm. Co. (Seoul, South Korea). All other chemicals were used without any further purification.

2.2. Preparation of hydrogels

PVA/chitosan hydrogels were obtained by the freezing–thawing (F–T) cycle. In brief, the solutions containing 10% (w/v) PVA, 1.5% (w/v) chitosan and minocycline (0 or 0.25%) were prepared in distilled water. The solutions with different proportions of PVA and chitosan (chitosan = 0, 5, 10, 20 and 30%) were mixed by vortexing for 1 h and poured into Petri dishes. They were then frozen at −20 °C for 18 h and then thawed at room temperature for 6 h for three consecutive cycles (Table 1) (Cascone et al., 1999; Huang and Yang, 2008).

2.3. Gel fraction

After three F–T cycles, the hydrogel samples were dried for 6 h at 50 °C under vacuum (W_o). They were next soaked in distilled water for 24 h up to a constant weight to remove the soluble parts. The gels were then dried again at 50 °C under vacuum (W_e). The gel fraction percentage was calculated by the following equation (Ajji et al., 2005).

$$\text{Gel fraction}\% = \left(\frac{W_e}{W_o}\right) \times 100,$$

where W_o and W_e are the weights of hydrogel samples dried for 6 h at 50 °C before and after soaking, respectively.

2.4. Swelling

The pieces of hydrogel samples (2 cm × 2 cm) were dried at 60 °C under vacuum for 12 h (W_a). They were then soaked in pH 7.4 phosphate buffer solution (PBS) at 37 °C (W_s). The swelling ratio (SR) was calculated using the following equation (Ajji et al., 2005; Kim et al., 2008).

$$\text{SR}\% = \left(\frac{W_s}{W_a}\right) \times 100;$$

where W_a and W_s are the weights of hydrogel samples dried for 12 h at 60 °C and soaked in PBS at 37 °C, respectively.

2.5. Water vapour transmission test

The water vapour transmission tests were performed using the JIS 1099A standard method (Huang and Yang, 2008). A round piece of hydrogel was mounted on the mouth of a cup (7 cm diameter) containing 50 g of $CaCl_2$ and placed in an incubator of 90% RH at 40 °C. The water vapour transmission rate (WVTR) was determined as follows:

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

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

2.6. Morphology observation

The internal structure of the samples was investigated by scanning electron microscopy (SEM, S-4100, Hitachi, Japan). The hydrogels were dehydrated by freeze-dryer and coated with platinum using an ion sputter (E-1030, Hitachi, Ltd., Japan) (Cascone et al., 1999).

2.7. Mechanical properties

The tensile strength and breaking elongation of hydrogels were determined using a tensile test machine (Instron 4464, UK). After three F–T cycles, the hydrogels were cut into a specific dog bone shape (6 cm long, 2 cm wide at the ends and 1 cm wide in the middle). The mechanical analysis was performed at a stretching rate of 20 mm/min with pre-load of 0.5 N to determine the maximum load for each matrix (Kim et al., 2008). The thickness of the hydrogel was measured with a digital calliper (CD-15CPX, Mitutoyo Co., Japan) before examination (Lin et al., 2006).

2.8. Release

One side of the hydrogel was attached to a Teflon frame instrument that was immersed into 500 ml distilled water as a dissolution medium at 37 °C. It was stirred at the paddle speed of 50 rpm for 48 h. At predetermined time intervals, 5 ml of the medium was withdrawn, diluted with the dissolution medium and filtered using a 0.45 μm syringe filter. The concentration of the drug was deter-

mined at 280 nm using a UV spectrophotometer (U-2800, Hitachi, Ltd., Japan) (Aoyagi et al., 2007; Kim et al., 2008).

2.9. Adsorption of protein onto hydrogel surface

The pieces of hydrogels cut into 2 cm × 2 cm were immersed in 4 ml of pH 7.4 PBS containing HSA and HPF proteins at 37 °C. After shaking at 100 rpm for 24 h, they were gently taken out and rinsed five times with PBS. They were placed in six wells containing an aqueous solution of 1% sodium dodecyl sulphate and shaken for 1 h to remove the protein adsorbed on the surface. The protein contents of each sample were measured using the BCA reagents. The absorbance at 562 nm was measured using a microplate reader (Molecular Devices Versa MAX Sunnyvale, CA, USA) (Kim et al., 2008; Huang and Yang, 2008).

2.10. In vivo wound healing test

Male SD rats weighting approximately 250–280 g were used to evaluate the *in vivo* wound healing test of hydrogels. All animal care and procedures were conducted according to the Guilding Priciples in the Use of Animals in Toxicology, as adopted in 1989 and revised in 1999 by the Society of Toxicology (SOT, 1999).

The rats were anesthetised by i.p. injection of Zoletil 50® (tiletamine/zolazepam) and the dorsal hair of each animal was shaved with an electric razor. After creating two full thickness wound areas (1.5 cm × 1.5 cm) by excising the dorsum, 70% ethanol was used for sterilisation. Each wound was covered with sterile gauze (control), the hydrogel without drug, the hydrogel with drug and the commercial product, respectively. All materials were fixed with an elastic adhesive bandage. All rats were separately kept in individual cages. At the 3rd, 6th, 9th, 12th and 15th days after the operation, each wound size was measured using a digital camera.

2.11. Histopathology

2.11.1. Histological process

The wounded area of skin containing dermis and hypodermis was sampled and crossly trimmed. All trimmed skins were fixed in 10% neutral buffered formalin. After paraffin embedding, 3–4 μm sections were prepared. Representative sections were stained with hematoxylin and eosin (H&E) for microscopic examination, or Masson's trichrome for collagen fibres (Burkatovskaya et al., 2006; Kim et al., 2000; Park et al., 2004).

2.11.2. Histomorphometry

The desquamated epithelium regions (mm), numbers of microvessels in granulation tissues (vessels/mm^2 of field), numbers of infiltrated inflammatory cells in granulation tissues (cells/mm^2 of field), percentages of collagen-occupied regions in granulation tissues (%/mm^2 of field) and thicknesses of central regions of granulation tissues (mm from epidermis to dermis) were measured on the histological skin samples using a digital image analyser (DMI-300, DMI, South Korea) (DMI, Korea), respectively (Quintanilha Ribeiro et al., 2008). Furthermore, re-epithelisation was also calculated as follows (Hirose et al., 2007):

$$\text{Re-epithelisation } (\%) = \left[\frac{A-B}{C}\right] \times 100,$$

where A, B and C are 'total length of the total wound (10 mm)', 'desquamated epithelium regions (mm)' and 'total wound (10 mm)', respectively.

2.11.3. Statistical analysis

Multiple comparison tests for different dose groups were conducted. Variance homogeneity was examined using the Levene test. If the Levene test indicated no significant deviations from variance homogeneity, the data were analysed by one-way ANOVA test and the least significant differences (LSD) multi-comparison test. In case of significant deviations from the variance, homogeneity was observed using the Levene test, a non-parametric comparison test and the Kruskal–Wallis H-test. Statistical analyses were conducted using SPSS for Windows (Release 14.0, SPSS Inc., USA). Moreover, to evaluate the comparable efficacy of gauze control and test materials, their changes were calculated as follows:

$$\text{Percentage changes compared with gauze control } (\%) = \left(\frac{A_T - A_C}{A_C}\right) \times 100,$$

where A_T and A_C are data of tested groups and gauze control, respectively.

3. Results and discussion

Three consecutive cycles of freezing and thawing led to the formation of an insoluble miscible and entangled PVA/chitosan hydrogel. These hydrogels were a matrix of physically cross-linked polymeric chains, non-cross-linked polymer and water. The influence of chitosan and drugs on the gel fraction percentage is shown in Fig. 1A. The greater the increase of chitosan was, the lower the decreased gel fraction was (Kim et al., 2008). The gel fraction in

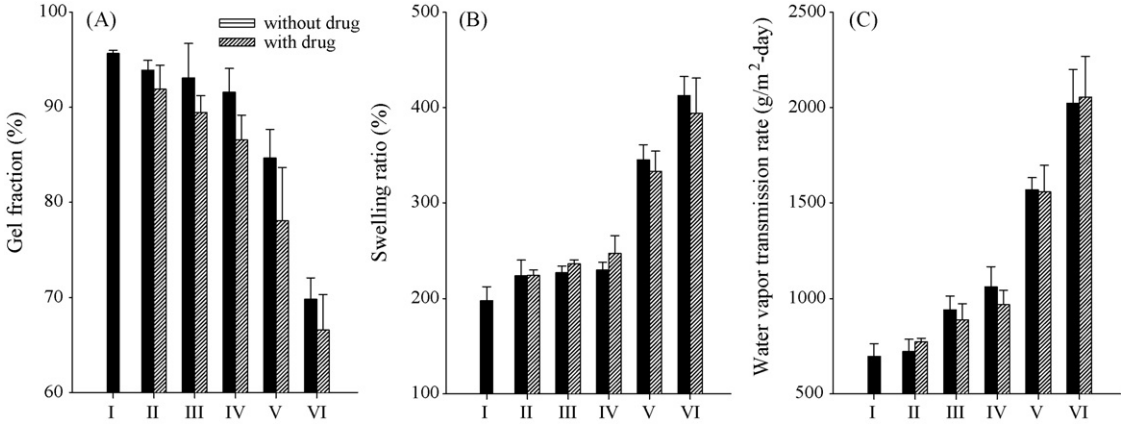


Fig. 1. Effect of chitosan and drugs on the gel fraction (A), swelling ratio (B) and WVTR (C). Each value represents the mean ± SD (n = 3).

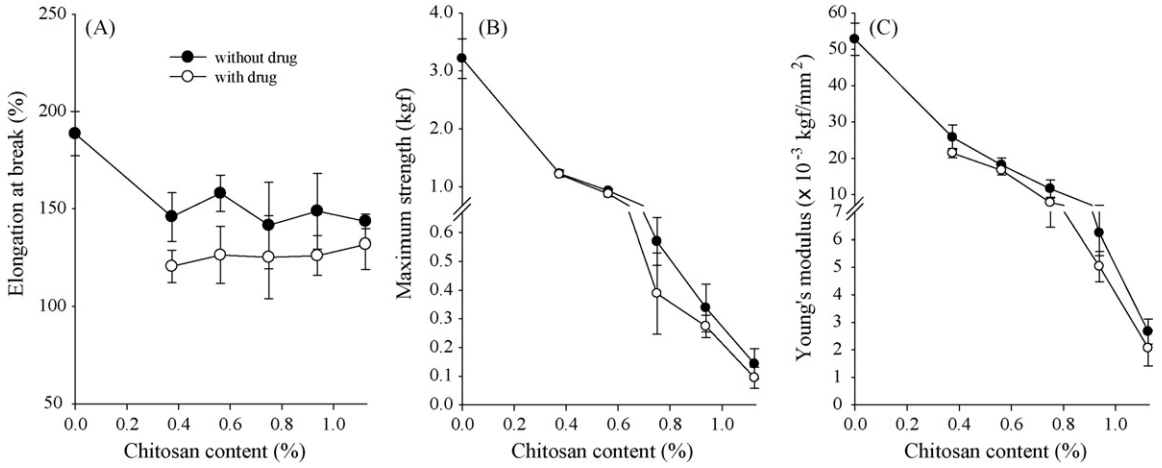


Fig. 2. Effect of chitosan and drug on the elongation at break (A), tensile strength (B) and Young's modulus (C). Each value represents the mean ± SD ($n = 3$).

the absence of chitosan was about 95% and relatively high, suggesting that PVA was almost completely cross-linked (Yokoyama et al., 1986). Thus, chitosan decreased the gel fraction of hydrogels. In particular, the hydrogel with 1.125% chitosan decreased to less than 70%. During F–T cycles, the cross-linking strength of chitosan was weaker than that of PVA, even if chitosan formed a cross-linking bond with PVA in the gel. Generally, as the gel fraction decreased, the strength and flexibility of the gel were weakened (Ajji et al., 2005; Kim et al., 2008). Chitosan could be used to control the gel fraction and strength of hydrogel because it reduced the cross-linking reaction between polymers in the gel. There was no significant difference in gel fraction of the hydrogel with or without drugs. Thus, the drugs dissolved in the gel hardly affected the gel fraction.

The swelling ability increased to more than 400% at 1.125% chitosan, as chitosan concentration increased (Fig. 1B). This indicated that chitosan produced a less cross-linked hydrogel than PVA did. Lesser cross-linked hydrogels tended to show a higher water uptake, because the highly cross-linked structure could not sustain much water within the gel structure (Balakrishnan et al., 2005; Choi et al., 1999). The drug hardly changed the swelling ability of the hydrogel.

Fig. 1C shows the effect of chitosan and drugs on the WVTR of the hydrogel. Queen reported that a water vapour transmission rate of 2000–2500 g/m^2 per day would provide an adequate level of moisture to prevent excessive dehydration and build up the exudates on the wound area (Queen et al., 1987). A higher WVTR dried the wound more quickly and produced scars. Moreover, a lower WVTR accumulated exudates, which might retard the healing process and increase the risk of bacterial growth (Kokabi et al., 2007). In this study, as chitosan increased, the WVTR of hydrogel also increased. The WVTR of the hydrogel with 1.125% chitosan was up to 2000 g/m^2 per day, which was an ideal value for wound dressing. Thus, it might provide an adequate level of moisture and build up the exudates on the wound area. Similarly, the hydrogel with drugs scarcely improved the WVTR compared with the hydrogel without drugs.

To investigate the influence of chitosan on the mechanical properties of the hydrogels, their tensile strength, elongation at break and Young's modulus was evaluated (Fig. 2). Chitosan hardly affected the elongation at break (Fig. 2A). However, it decreased the tensile strength (Fig. 2B) and Young's modulus of the hydrogel (Fig. 2C). When chitosan was blended with PVA, the cross-linking density of the hydrogel was decreased (Rosiak et al., 2001). Our results suggested that chitosan produced less stiff and more elastic hydrogels (Cascone et al., 1999; Kim et al., 2006). Similarly, there was no significant difference in the mechanical properties of the hydrogel with or without drugs.

As shown in Fig. 3A–C, the higher the chitosan, the greater the porous size of the hydrogel. Our results suggested that the entangling between PVAs was reduced by chitosan (Tang et al., 2007). Chitosan could decrease the network densities because it induced relatively entangled porous sizes and perturbed the stable three-dimensional polymer network (Li et al., 2008). However, the drug incorporated in the hydrogel did not significantly influence the porosity (Fig. 3B vs. D; C vs. E).

There were no significant differences in the release of drugs from all minocycline-loaded hydrogels (Fig. 4A). Therefore, chitosan hardly affected the release of drugs. To understand the release mechanism of drugs from the hydrogels, we described the release rate using the following equations:

$$\frac{M_t}{M} = kt^n$$

$$\log\left(\frac{M_t}{M}\right) = \log k + n\log(t)$$

where M_t/M is the fraction of released drugs at time t, k is a characteristic constant of the hydrogel and n is an indication of the release mechanism.

As the k value became higher, the release occured faster. The n value of 1 corresponded to zero-order release kinetics, $0.5 < n < 1$ meant a non-Fickian release model and $n = 0.5$ indicated Fickian diffusion (Higuchi model) (Peppas, 1985). From the plot of $\log(M_t/M)$ vs. $\log(t)$ (Fig. 4B), the kinetic parameters n and k were calculated. Table 2 shows that most n values were close to 0.5, suggesting that the drug was released from hydrogels by Fickian diffusion through the extramicellar aqueous channels of the gel matrix (Kim et al., 2006). Furthermore, the similar n values indicated that chitosan scarcely affected the release mechanism. As mentioned above in Figs. 1B and 3, because chitosan increased the swelling ability of hydrogels and decreased the network densities, it was predicted

Table 2
Release kinetic parameters.

Hydrogel	Release exponent, n	Kinetic constant, k (%/h^n)	Correlation coefficient, r
II	0.539	1.540	0.973
III	0.525	1.563	0.961
IV	0.317	1.564	0.954
V	0.541	1.558	0.961
VI	0.531	1.565	0.964

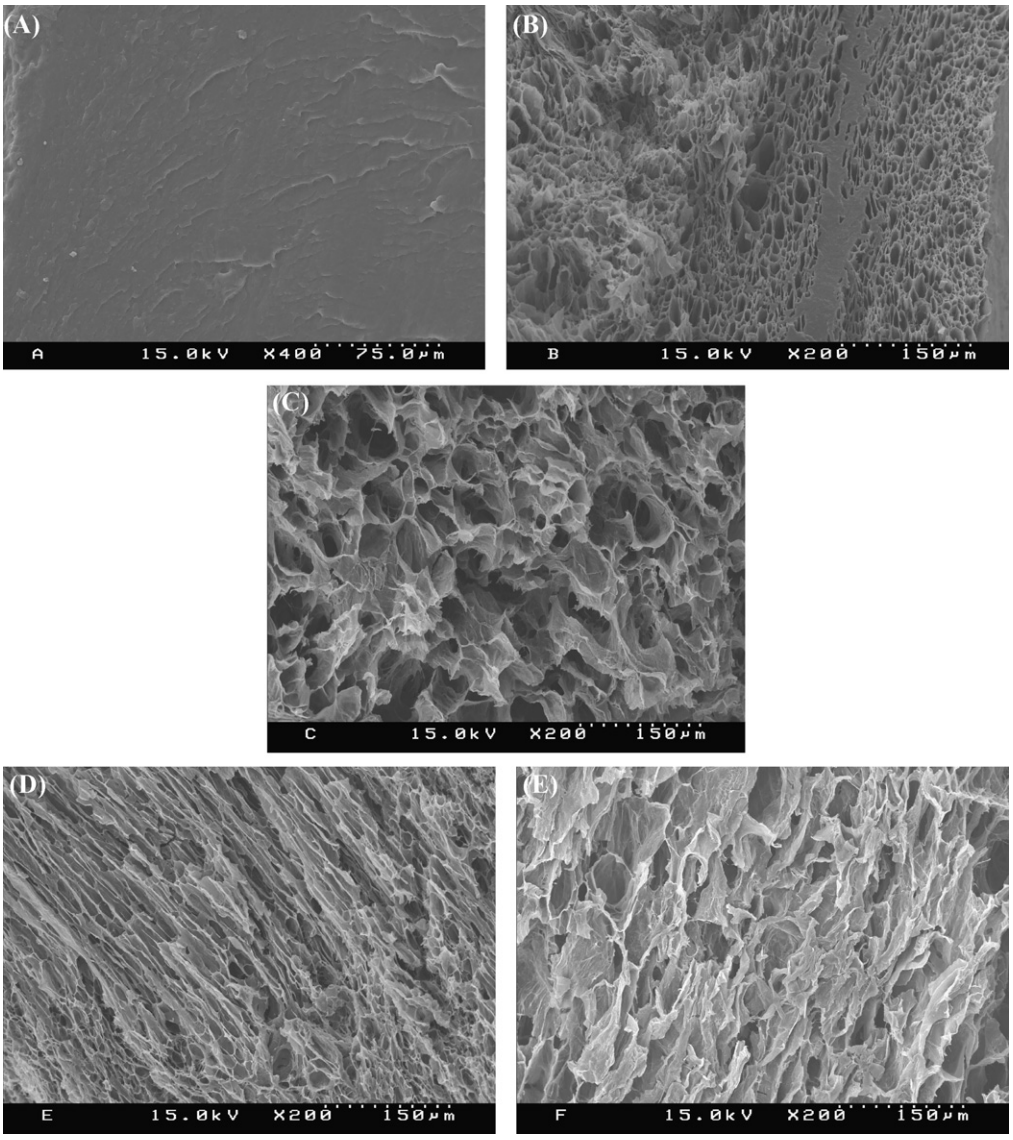


Fig. 3. SEM micrographs: (A) hydrogel with only PVA, (B) hydrogel with 0.375% chitosan and no drug, (C) hydrogel with 0.75% chitosan and no drug, (D) hydrogel with 0.375% chitosan and 0.25% drug and (E) hydrogel with 0.75% chitosan and 0.25% drug.

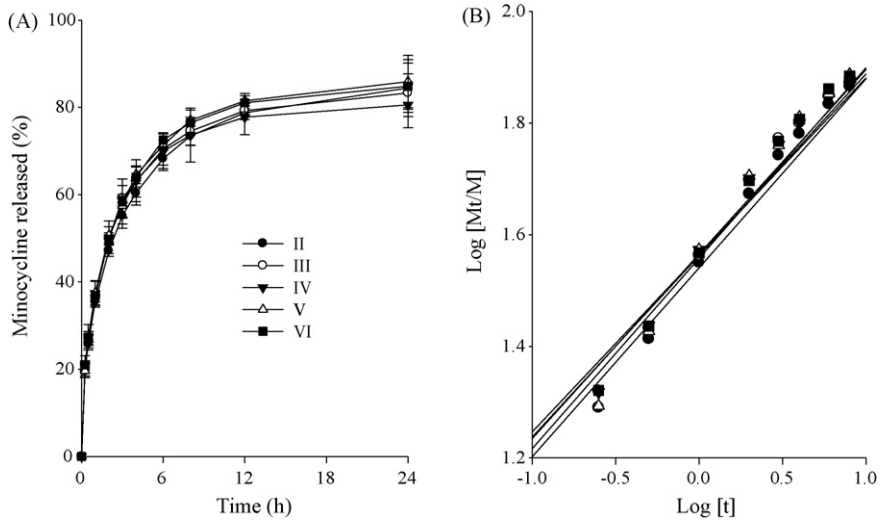


Fig. 4. Effect of chitosan on the drug release (A) and release kinetics (B). Each value represents the mean ± SD ($n = 6$).

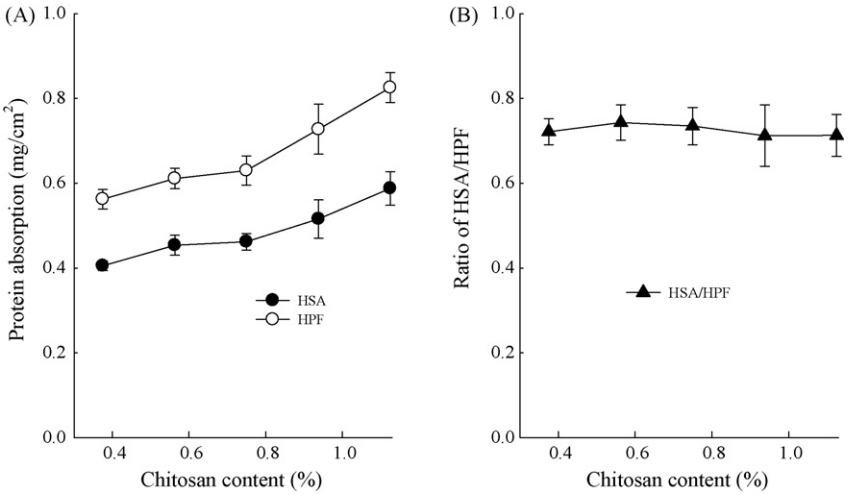


Fig. 5. Effect of chitosan on the protein absorption (A) and the HSA/HPF ratios (B). Each value represents the mean ± SD ($n = 3$).

that chitosan improved the release of drugs from hydrogels. However, the similar k values indicated that chitosan hardly affected the release of drugs from hydrogels. Our results suggested that the cross-linked interaction of PVA by chitosan were not decreased to improve the release of drugs from hydrogels, even if chitosan increased swelling ability of hydrogels (Aoyagi et al., 2007; Jain et al., 2007; Orti et al., 2000).

The blood compatibility of the hydrogel was evaluated by the amount of plasma protein adsorbed onto the hydrogel surface. When foreign material was placed in contact with blood, the adsorption of protein onto the surface occurred, leading to platelet adhesion and activation (Coleman et al., 1982; Lin et al., 2006). Because the albumin adsorption on the synthetic surfaces could inhibit platelet activation, it did not promote clot formation. Generally, as the albumin/fibrinogen adsorption ratio was higher, the number of adhering platelets was lower (Dion et al., 1993). Fig. 5 shows the albumin (HSA) and fibrinogen (HPF) adsorptions onto the PVA/chitosan membranes and the albumin/fibrinogen adsorption ratio. The adsorption of both HSA and HPF increased as chitosan increased (Lin et al., 2006; Wang et al., 2003). However, the HSA/HPF adsorption ratio hardly changed as chitosan increased. Therefore, chitosan did not significantly affect the adhesion of platelets to artificial surfaces.

From these findings, the wound dressing developed with PVA and chitosan was more swellable, flexible and elastic than that with only because of its cross-linking interaction with PVA. The hydrogel composed of 5% PVA, 0.75% chitosan and 0.25% drug was selected as a formulation of minocycline-loaded wound dressing for further study.

To estimate the wound healing effect of the hydrogel for the acceleration of wound repair, the hydrogels were applied to wound spots in the rat dorsum. Fig. 6 shows the macroscopic appearance of wounds treated with a sterile gauze, hydrogel with drugs, hydrogel without drugs and conventional products at various days of post-operation. In this experiment, a sterile gauze was used as a control. The hydrogel without or with drugs was composed of 87% PVA, 0.75% chitosan and no drug, or 0.25% of drug, respectively. Each wound was observed for a period of 3, 6, 9, 12 and 15 days post-operation. All rats survived throughout the post-operative period until sacrifice. There was no evidence of necrosis. At 3 days post-operation, little discrete inflammation was observed in all rats. The conventional product and the hydrogels with and without drugs induced no infection or contraction of the wound, whereas the control group showed a hemorrhagic and scabbed wound spot. At 6 days post-operation, the gauze control showed hemorrhage by second damage. However, the hydrogels hardly induced hemorrhage. In addition, epithelialisation acceleration and keratinocyte migration occurred more easily in these hydrogels (Winter, 1962). From 9 days post-operation, the majority of the wounds appeared to be healed and were almost completely sealed. The relative size reduction of the wounds treated with various materials is illustrated in Fig. 7. The wound size reduction was calculated as follows:

$$\text{Wound size reduction } (\%) = \left(\frac{A_o - A_t}{A_o}\right) \times 100,$$

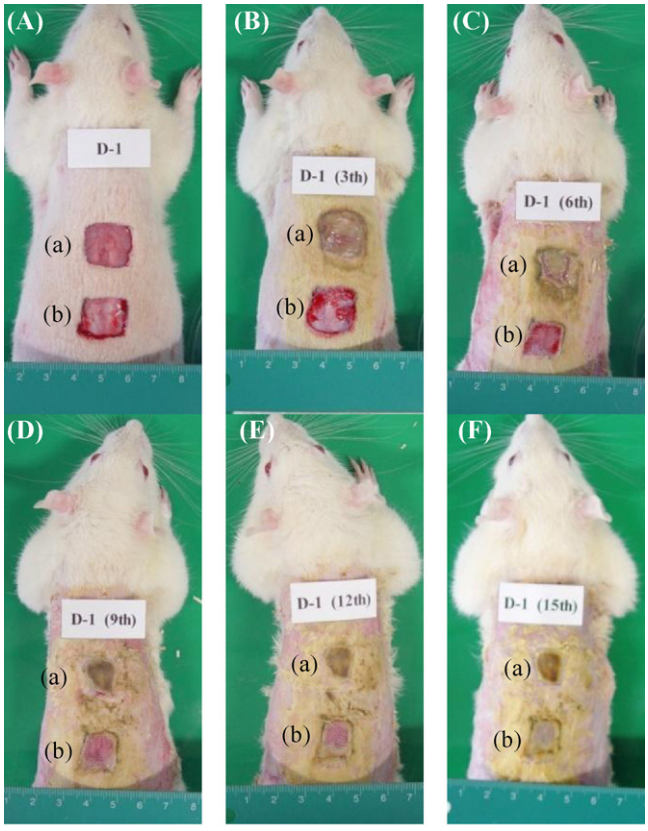
where A_o and A_t are the wound sizes at initial time and time t, respectively. The wound size was surveyed using the Adobe® Acrobat® 7 program (Balakrishnan et al., 2005; Kim et al., 2008).

Until 6 days, there were no significant differences in wound size reduction. From 9 days post-operation, the hydrogel with and without drugs significantly decreased the wound size compared with the control. Furthermore, from 12 days, the hydrogel with and without drugs showed a significantly greater wound size reduction than the conventional product did. In particular, about a 80–90% wound size reduction was seen for the hydrogels at 15 days. Chitosan has been reported to accelerate wound healing (Cho et al., 1999).

Table 3
Histomorphometrical values.

Groups histomorphometry	Gauze (control)	Conventional product	Hydrogel without drug	Hydrogel with drug
Lengths of desquamated epithelial regions (mm)	7.74 ± 3.03	0.49 ± 1.08[a]	9.14 ± 0.99	0.15 ± 0.35[a]
In the granulation tissues				
Numbers of microvessels (vessels/1 mm² of field)	4.40 ± 1.67	20.80 ± 6.72[a]	3.20 ± 2.28	23.40 ± 8.99[a]
Numbers of inflammatory cells (cells/1 mm² of field)	2106.00 ± 934.45	250.00 ± 217.17[a]	2447.00 ± 527.81	179.00 ± 227.74[a]
Percentages of collagen tissue occupied regions (%/1 mm² of field)	36.73 ± 8.96	66.21 ± 10.07[a]	33.41 ± 4.50	75.81 ± 9.44[a]
Thickness of central regions (mm, from the epidermis to dermis)	2.56 ± 0.58	2.12 ± 0.47	3.40 ± 0.35[a]	2.14 ± 0.36

Each value represents the mean ± SE ($n = 6$).
[a] $p < 0.01$ compared with gauze (control).



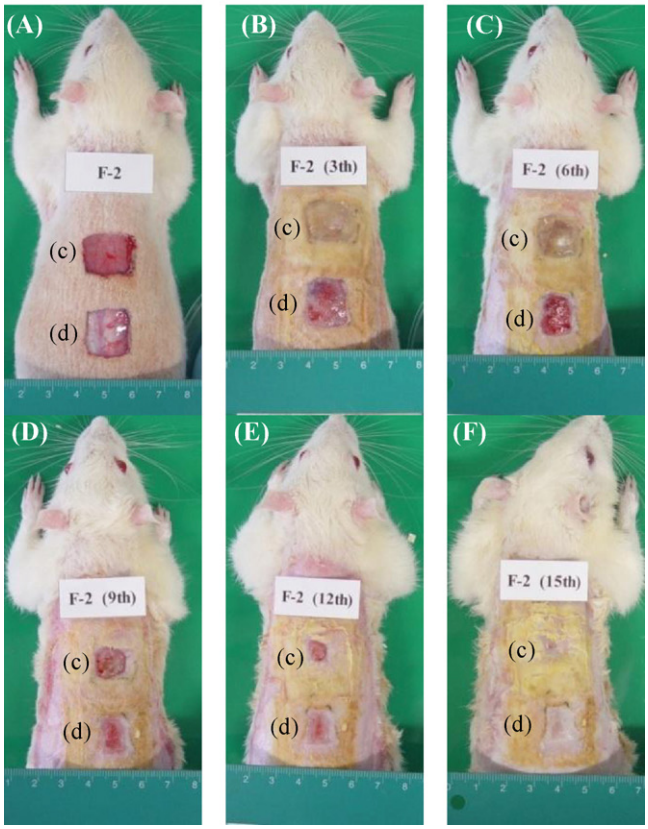


Fig. 6. Photographs of wounds treated with (a) hydrogel with 0.75% chitosan and 0.25% drug, (b) sterile gauze, (c) hydrogel with 0.75% chitosan and no drug, (d) conventional product at (A) 0 day, (B) 3 days, (C) 6 days, (D) 9 days, (E) 12 days and (F) 15 days of post-operation.

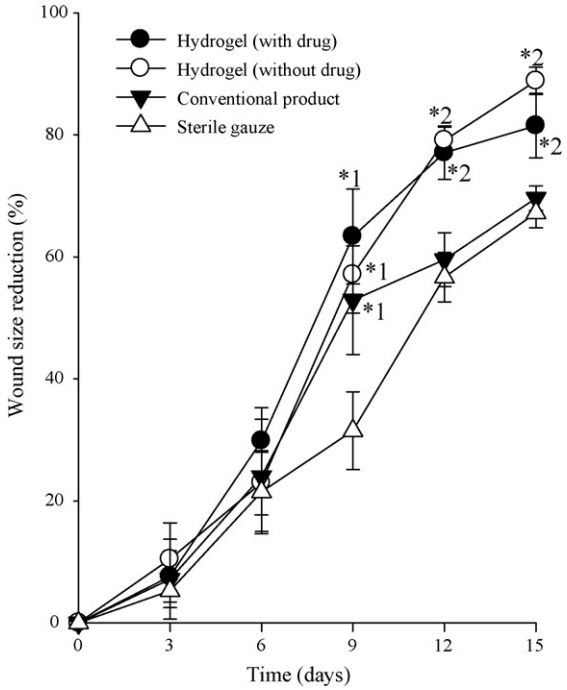


Fig. 7. Size reduction of wound treated with sterile gauze, hydrogel with 0.75% chitosan and 0.25% drug, hydrogel with 0.75% chitosan and no drug and conventional product in rat dorsum. Each value represents the mean ± SD ($n = 6$). $^{*1}p < 0.05$ compared with the sterile gauze. $^{*2}p < 0.05$ compared with the sterile gauze and the conventional product.

By contrast, there were no significant differences in wound size reduction between the hydrogel with and without drugs. Thus, irrespective of the addition of drugs, the PVA/chitosan-based hydrogel greatly improved wound healing.

The conventional product and hydrogel with drugs gave significantly higher re-epithelisation rates than did the gauze control and the hydrogel without drugs (Fig. 8). However, there was no significant difference between the gauze control and the hydrogel without drugs.

The changes on the desquamated epithelium regions, numbers of microvessels, infiltrated inflammatory cells, percentages of collagen-occupied regions and thicknesses of central regions of granulation tissue are listed in Table 3. In addition, the representative histological profiles of test groups are shown in Fig. 8. The hydrogel with drugs and conventional product gave more extended re-epithelisation of desquamated epithelial regions (A, D, G, J; asterisk) and less inflammatory cell infiltrations in granulation tissues (B, E, H, K) compared with the gauze control. Furthermore, compared with the gauze control, more numerous collagen proliferations (C, F, I, L; green colors) and neovasculations (B, E, H, K; arrows) were detected in them (for interpretation of the references to color in this sentence, the reader is referred to the web version of the article). They significantly decreased the infiltrated inflammatory cells and desquamated epithelial regions compared with the gauze control (Table 3, Fig. 8). In addition, the numbers of microvessels and collagen tissues in granulation tissues were significantly increased in them. However, the hydrogel without drugs (G–I) slightly delayed the wound healing histopathological changes compared with the gauze control (A–C) (Table 3, Fig. 8).

Wound healing is a fundamental response to tissue injury. The healing process can be related to inflammation, leading to epithelialisation, the formation of granulation tissue and tissue remodelling (Evans, 1980). It involves overlapping steps of inflammation, cell migration and proliferation, neovascularisation,

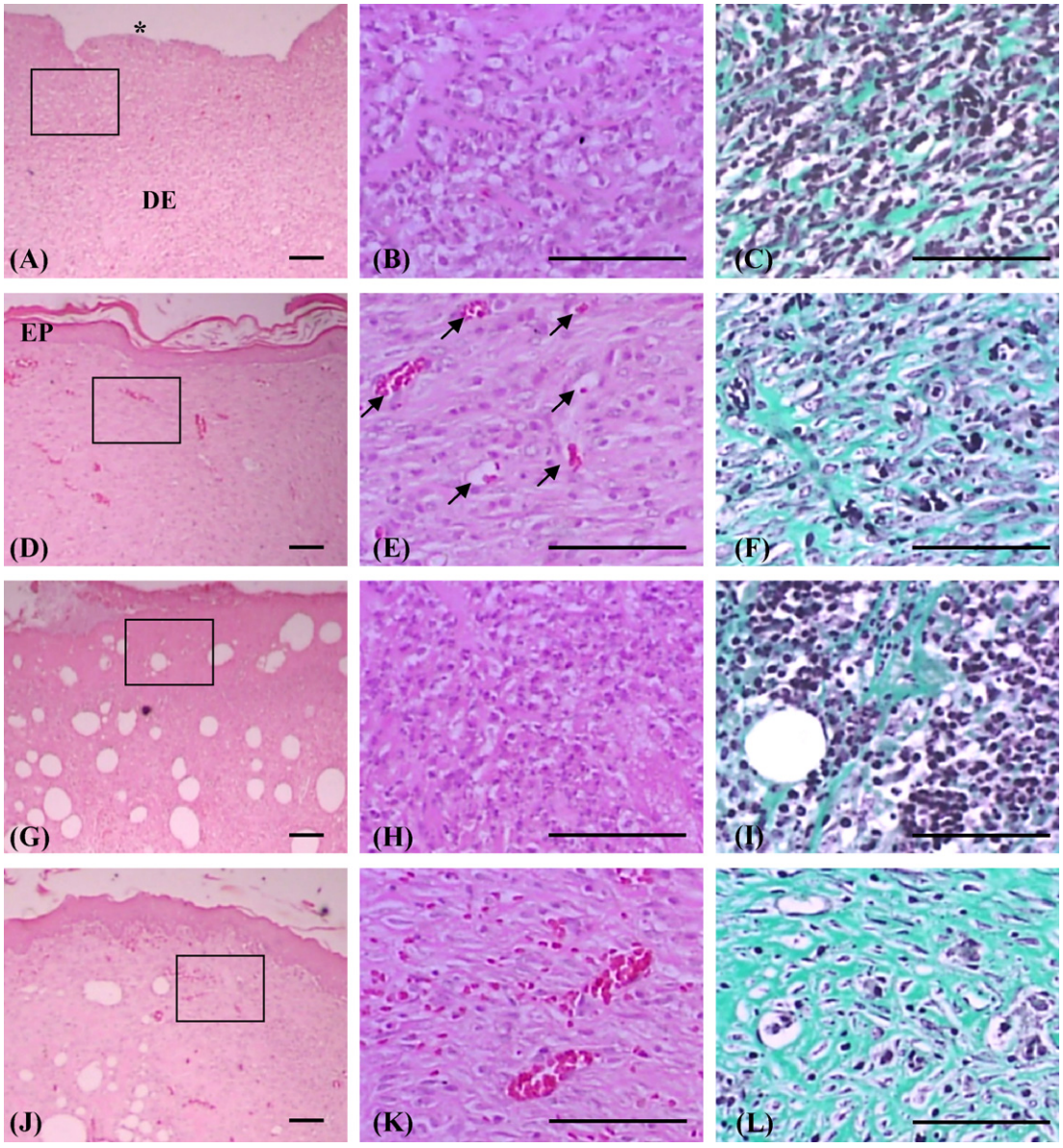


Fig. 8. Representative histopathological profiles of skin wounds (granulation tissues) of gauze control (A–C), conventional product (D–F), hydrogel with 0.75% chitosan and no drug (G–I), and hydrogel with 0.75% chitosan and 0.25% drug (J–L). DE, dermis; EP, regenerated epithelium; squares mean the enlarged areas in right columns; A, B, D, E, G, H, J and K: H&E stain; C, F, I and L: Masson's trichrome stain, scale bars = 80 μm.

extracellular matrix production and remodelling (Froget et al., 2003). Inflammation followed by tissue repair is a complex physiological process aimed at restoring normal function after infection or wounding (Martin, 1997). The most important criterion is the facilitated contraction and reconstruction with less scar formation (Sugihara et al., 2000). Angiogenesis was also treated as an important criterion in wound healing, especially in the later stages of tissue repair as a process essential to restoring tissue damaged by injury and inflammation (Halper et al., 2003). In this study, the hydrogel with drugs facilitated more favourable re-epithelisation, microvessel formation and reconstruction of skin tissue than did the conventional product. It contained more collagen tissues and less inflammatory cells compared with the conventional product. By contrast, the hydrogel without drugs hardly influenced or delayed the reconstruction of histological skin architectures. Thus, in histological examination, the hydrogel with drugs significantly improved wound healing compared with the hydrogel without drugs because of the potential healing effect of minocycline (Gehrig and Warshaw, 2008).

PVA is a biodegradable and nontoxic polymer. Because PVA and chitosan showed a high aqueous solubility, they needed cross-linking to be applied to the human body as a wound dressing system. The PVA hydrogels prepared by the F–T method formed physically cross-linked polymer chains containing uncross-linked polymer and water. These gels were non-toxic, non-carcinogenic and biocompatible. In this work, the cross-linked hydrogels were prepared at various proportions of chitosan to PVA. Chitosan decreased the gel fraction and tensile strength of the hydrogels, but increased the swelling ability, WVTR, elasticity and porosity of hydrogels. The drug hardly affected the gel properties of hydrogels. Thus, the minocycline-loaded wound dressing with PVA and chitosan was more swellable, flexible and elastic because of the relatively weak cross-linking interaction between PVA and chitosan. Furthermore, in healing tests, the PVA and chitosan-based hydrogel greatly improved wound healing unlike PVA and sodium alginate-based hydrogels (Kim et al., 2008a,b). It was due to antifungal activity of chitosan (Cho et al., 1999; Chung et al., 1994). In histological examination, the minocycline-loaded hydrogel gave a

greater healing effect than did the hydrogel without drugs because of the potential healing effect of minocycline. Compared to conventional product, the minocycline-loaded wound dressing provided an adequate level of moisture and build up the exudates on the wound area. It gave excellent elasticity and enhanced wound healing (Aoyagi et al., 2007; Gehrig and Warshaw, 2008).

4. Conclusion

The minocycline-loaded wound dressing developed with PVA and chitosan was more swellable, flexible and elastic because of its cross-linking interaction with PVA. The hydrogel composed of 5% PVA, 0.75% chitosan and 0.25% drug significantly improved the wound healing effect compared with the gauze control, the hydrogel without drugs and the conventional product. Thus, it is a potential wound dressing with excellent forming and enhanced wound healing.

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