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Development of polyvinyl alcohol–sodium alginate gel-matrix-based wound dressing system containing nitrofurazone

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ABSTRACT

Polyvinyl alcohol (PVA)/sodium alginate (SA) hydrogel matrix-based wound dressing systems containing nitrofurazone (NFZ), a topical anti-infective drug, were developed using freeze–thawing method. Aqueous solutions of nitrofurazone and PVA/SA mixtures in different weight ratios were mixed homogeneously, placed in petri dishes, freezed at −20 °C for 18 h and thawed at room temperature for 6 h, for three consecutive cycles, and evaluated for swelling ratio, tensile strength, elongation and thermal stability of the hydrogel. Furthermore, the drug release from this nitrofurazone-loaded hydrogel, in vitro protein adsorption test and in vivo wound healing observations in rats were performed. Increased SA concentration decreased the gelation%, maximum strength and break elongation, but it resulted into an increment in the swelling ability, elasticity and thermal stability of hydrogel film. However, SA had insignificant effect on the release of nitrofurazone. The amounts of proteins adsorbed on hydrogel were increased with increasing sodium alginate ratio, indicating the reduced blood compatibility. In vivo experiments showed that this hydrogel improved the healing rate of artificial wounds in rats. Thus, PVA/SA hydrogel matrix based wound dressing systems containing nitrofurazone could be a novel approach in wound care.

1. Introduction

A large number of dressings are currently used in the management of burns, split graft donor sites, chronic ulcers, decubitus ulcers, and so on (Dyson et al., 1991; Eaglstein and Pittsburgh, 1985; Suzuki et al., 1997; Tanihara et al., 1998). There are two kinds of dressings: dry type and wet type. It has been reported that healing with a wet environment is faster than that with a dry environment (Winter, 1962). In recent years, hydrogels have received considerable attention to be used as specific absorbents in wound dressing materials. Thus, a number of polymers with superabsorbent properties have been developed for clinical applications, such as liquefaction and removal of scar, treatment of leg ulcers, pressure sores, and prevention of tissue deterioration in patients with restricted mobility.

Polyvinyl alcohol (PVA) has several useful properties including non-toxicity, biocompatibility, high hydrophilicity, fiber/film forming ability, and the chemical and mechanical resistance. It has been widely commercialized and studied in the chemical and medical industries for the productions of fibers, films, coatings, cosmetics, pharmaceuticals, and so on (Yeo et al., 2000). PVA hydrogels produced by using the freezing–thawing technique form a matrix of physically crosslinked polymeric chains containing uncrosslinked polymer and water. The use of freeze–thawed PVA hydrogels has been explored for biomedical and pharmaceutical applications. These gels are non-toxic, non-carcinogenic, have good biocompatibility, and have desirable physical properties such as rubbery nature and high degree of swelling in water (Peppas and Stauffer, 1991). PVA must be crosslinked if it is to be used in biodegradable materials. PVA hydrogel has excellent transparency and is smooth as membrane, and it is also biologically inactive and bio-compatible. It has attracted much attention to be widely used as a good material for temporary skin covers or burn dressings (Peppas and Scott, 1992).

Alginate derived from brown algae is an anionic linear polysaccharide composed of 1,4-linked β-D-mannuronates residues and 1,4-linked α-L-guluronates in varying proportions (Rees and Welsh, 1997). Alginate is hydrophilic, biocompatible, and relatively economical. It has been widely used in medical application such as wound dressings, scaffolds for hepatocyte culture and surgical or dental impression material, even if the allergic reaction to skin

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has been occurred (Ng and Cheng, 2007; Patel, 1993). They have been successfully applied to cleanse a wide variety of secreting lesions. The high absorption is achieved via strong hydrophilic gel formation. This limit wound secretions and minimizes bacterial contamination. Alginate fibers trapped in a wound are readily biodegraded (Gilchrist and Martin, 1983). Alginate dressings maintain a physiologically moist microenvironment that promotes healing and the formation of granulation tissue. Alginate can be rinsed away by saline irrigation, thus the removal of the dressing does not interfere with healing granulation tissue. This makes dressing changes virtually painless. Alginate dressings are very useful for moderate to heavily exuding wounds (Motta, 1989). The importance of polymer blending has been increased in recent years because of the preparation of the polymeric materials with desired properties, low basic cost, and improved process ability. In this study, we attempted to use the sodium alginate as a component of PVA hydrogel for wound dressing.

2. Materials and methods

2.1. Materials

PVA (typical average M_w = 146,000–186,000; +99% hydrolyzed), sodium alginate, nitrofurazone (5-nitro-2-furfural semicarbazone), human serum albumin (HSA) (M_w = 66 kD, albumin: 97.31%) and human plasma fibrinogen (HPF) (M_w = 341 kD, clottable proteins > 95%) were purchase from Sigma–Aldrich, Kanto Chemical Co. (Tokyo, Japan), Fluka Co. (Germany) and Calbiochem Co. (Germany), respectively. All other chemicals were used without any further purification.

2.2. Preparation of hydrogels

PVA/SA hydrogels were obtained by freezing–thawing (F–T) cycle (Peppas and Stauffer, 1991). Solutions containing 10% (w/v) PVA and 3% (w/v) SA and nitrofurazone were prepared in deionized water. Different proportions of PVA and SA (SA = 0, 5, 10, 20 and 30%) solutions were mixed by vortexing for an hour, and the calculated amounts of this mixture were poured in petri dishes, followed by freezing at −20 °C for 18 h and thawing at room temperature for 6 h, for three consecutive cycles.

2.3. Determination of gel fraction

After three F–T cycles, the samples were dried for 6 h in 50 °C in an oven (W_0), then soaked in distilled water for 24 h up to a constant weight (equilibrium swelling W_s) in order to remove the soluble parts. The gels were then dried again in 50 °C oven (W_e). The gelation% was then calculated by the following equation.

$$\text{Gelation}(\%) = \frac{W_e}{W_0} 100.$$

2.4. Determination of swelling ratio

To measure the swelling behavior, hydrogel samples were cut into 2 cm × 2 cm pieces and dried at 50 °C in an oven for 1 h and their dry weights (W_e) were immediately measured, then they were soaked in PBS maintained at 37 °C and their weights (W_s) were determined at specific time points and the swelling ratio (SR) was calculated using the following formula:

$$\text{SR}(\%) = \frac{W_s}{W_e} 100.$$

Changing of 20% (w/w) sample's weight was also observed for 30 min. To minimize error caused by surface water, weights after immediate soaking (W_{si}) were also taken. The swelling ratio was then determined according to the following formula (Balakrishnan et al., 2005; Choi et al., 1999).

$$\text{SR}(\%) = \frac{W_s - W_{si}}{W_e} 100.$$

2.5. Determination of the 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 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. The thickness of each individual hydrogel was also measured (Alvaro Antonio Alencar et al., 2003; Lin et al., 2006; Wu et al., 2001).

2.6. Thermal analysis properties

The thermal analysis properties of hydrogels were determined using differential scanning calorimeter (DSC) (TA, USA). The samples were heated from room temperature to 300 °C. They were annealed for 2 min at this temperature and then cooled to 0 °C at the rate of 10 °C/min, and finally heated to 200 °C at a heating rate of 20 °C/min to obtain the crystallization temperature (T_c) and melting temperature (T_m). All the DSC measurements proceeded under the nitrogen flow of 10 ml/min. To determine the thermo-stability of blends, thermo gravimetric analysis (TGA) (TA, USA) was used with samples heated from 25 to 500 °C at a heating rate of 10 °C/min. Then, change of weights was observed (Lin et al., 2006).

2.7. Adsorption of protein onto hydrogel surface

Pieces of hydrogel membrane cut into 2 cm × 2 cm were immersed in 4 ml of pH 7.4 phosphate buffer solution (PBS) at 37 °C containing HSA and HPF proteins, and shaken at 100 rpm for 24 h. Samples were then gently taken out and rinsed five times with PBS, placed in six wells containing aqueous solution of 1% sodium dodecyl sulfate (SDS) and shaken for 1 h at room temperature to remove the protein adsorbed on the surface. The protein contents of the each sample were then measured using the Bradford reagent. The absorbance at 570 nm was measured using an ultraviolet spectrometer. The calibration curve was prepared by measuring varying protein concentrations in SDS solution (Alvaro Antonio Alencar et al., 2003; Lin et al., 2006).

2.8. Dissolution test

The drug release from hydrogel was measured by using Teflon frame instrument, as illustrated in Fig. 1. Only one side of sample was attached to the Teflon frame instrument that was immersed into 400 ml distilled water at 37 °C as the dissolution medium and stirred at the paddle speed of 50 rpm. One milliliter of the sample was withdrawn from the medium at various time intervals. The concentration of drug was determined by high-performance liquid chromatograph (PU-987 pump and UV-975 UV detector, Jasco). 50 μl of the samples were injected into the column (5 μm particle size, 4.6 mm × 150 mm, Inertsil C18, GL science) with a UV detector at 365 nm. The mobile phase was acetate buffer (pH 4.6)-acetonitrile (75:25, v/v) at 1 ml/min (Chung et al., 2003; Draisci et

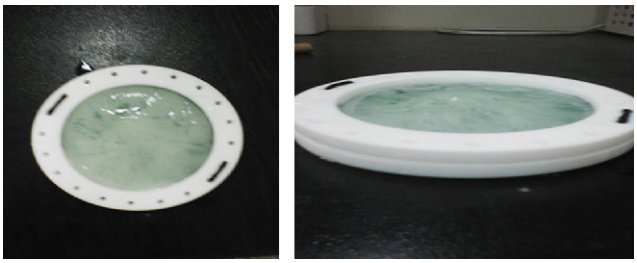

Fig. 1. Teflon® framed dissolution plates.

al., 1997; Peppas and Scott, 1992; Takamura et al., 1992; You et al., 2004).

2.9. In vivo wound healing test

Male SD rats weighting approximately 250–300 g was used to evaluate wound healing characteristics of hydrogels. The dorsal hair of rats was shaved and the animals were anesthetized with Zoletil50® (Tiletamine/Zolazepam)/Rompun® (Xylazine) cocktail by IP injection. Two full thickness skin wounds of 1.5 cm × 1.5 cm area were prepared by excising the dorsum of rats and disinfected using 70% ethanol. The excised wounds were covered with the ethylene oxide gas sterilized PVA/SA wound dressings (2 cm × 2 cm) containing nitrofurazone (control sample contained no drug) and fixed with elastic adhesive bandage (Soft cloth tape®, 3 M). After the experiment, rats were sacrificed by excess diethyl ether on 5, 10 and 15 days after surgery. The wounds were grossly examined and photographed for characteristics evaluation (Balakrishnan et al., 2005; Suzuki et al., 1999).

2.10. Histopathology

For histopathological study, the skins including the entire wound with adjacent normal skin were excised and fixed in 10% buffered formalin. The specimen included the dermis and the subcutaneous tissue. Excised wound sites fixed in formalin were processed and embedded in paraffin, and sections of 3–5 μm were stained with hematoxylin and eosin (Burkatovskaya et al., 2006; Kim et al., 2000; Park et al., 2004).

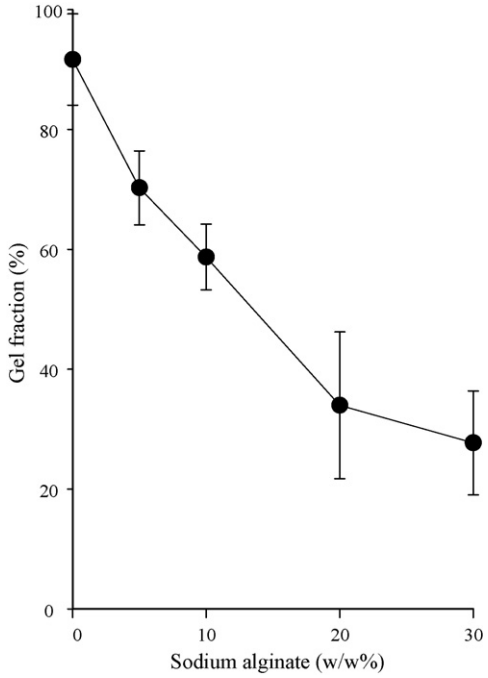

Fig. 2. Effect of SA on gel fraction. Data are expressed as mean ± S.D. (n = 3).

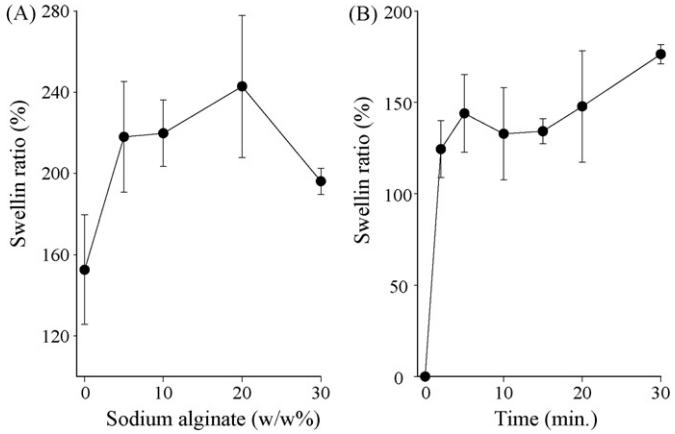

Fig. 3. Effect of SA on maximum swelling ratio of hydrogel dressings (A) and swelling ratios of 20% SA-loaded hydrogel dressings with respect to time (B). Data are expressed as mean ± S.D. (n = 3).

3. Result and discussion

3.1. Physicochemical properties

Freeze–thawed solution leads to the formation of insoluble hydrogel. The influence of SA concentration on gel fraction (%) was illustrated in Fig. 2. Generally, the lower gel fraction was, the weaker strength and less flexibility of gel was. The gel fraction in absence of SA was about around 90% and relatively high, suggesting that PVA was almost completely crosslinked (Yokoyama et al., 1986). Gel fraction decreased to less than 27% at 30% SA concentration with increasing SA concentration. In addition to acting as a wound healer, SA also reduces the crosslinking reaction and consequently the gelation process. Furthermore, in F–T cycle, the crosslinking strength of SA was weaker than that of SA, even though SA formed crosslinking in the gel. This effect can be utilized o control the gel fraction of hydrogel.

Fig. 3(A) shows the maximum swelling ability of the hydrogel dressings versus the SA proportion. In our preliminary swelling study, when the hydrogels were soaked in immersion liquid for

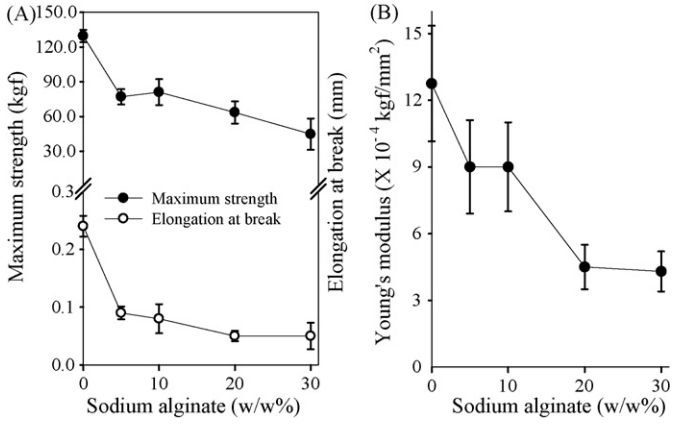

Fig. 4. Effect of SA on maximum strength and elongation at break (A), and Young's modulus (B). Data are expressed as mean ± S.D. (n = 3).

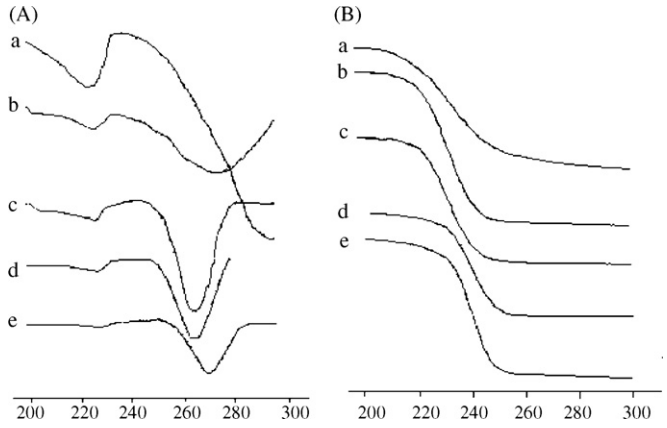

Fig. 5. DSC (A) and TGA (B) thermogram of PVA and PVA/SA mixed hydrogels: (a) SA 0% (only PVA); (b) SA 5%; (c) SA 10%; (d) SA 20%; (e) SA 30%.

30 min, very small amounts of SA were dissolved. Thus, the dissolved amounts of SA hardly affected the swelling test. It can be seen that the maximum swelling ability increases with increased SA proportion up to a certain limit then decreased. SA has better swelling ability, because it does not crosslink and is soluble in water. Lowly crosslinked hydrogels tended show higher water uptake ability, since the highly crosslinked structure could not sustain much water within gel structure (Balakrishnan et al., 2005; Choi et al., 1999). Swelling ability increased with increasing SA proportion. Fig. 3(B) shows the swelling kinetics of 20% hydrogel dressings. At the first stage of the curve, swelling rate was very high, and the water could be absorbed easily into the hydrogel. It can be seen that swelling increased after decreasing, due to the loss of SA because of its solubility.

To investigate the influence of SA on the mechanical properties of PVA/SA hydrogel, tensile strength and elongation at break were evaluated. Both the maximum strength and elongation at break decreased with increasing SA portions (Fig. 4A). Furthermore, the maximum strength at break gave the same pattern to elongation at break. Thus, our results suggested that sodium alginate weakened the break elongation of hydrogels, resulting in decreasing the maximum strength. The decrease of the tensile strength was believed

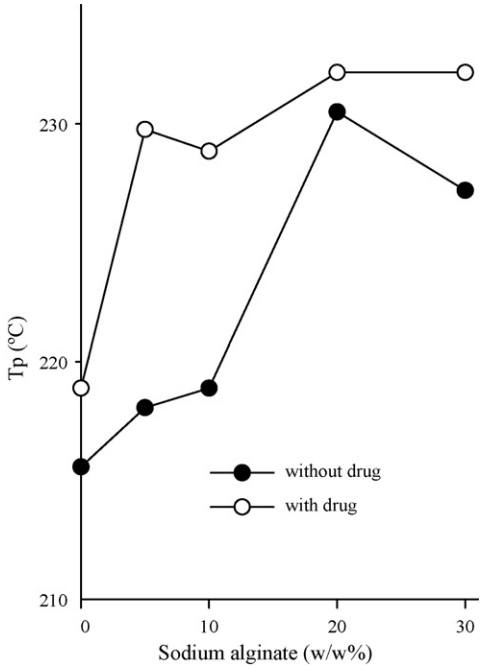

Fig. 6. Effect of SA on the T_p value of mixed hydrogels. Data are expressed as mean ± S.D. (n = 3).

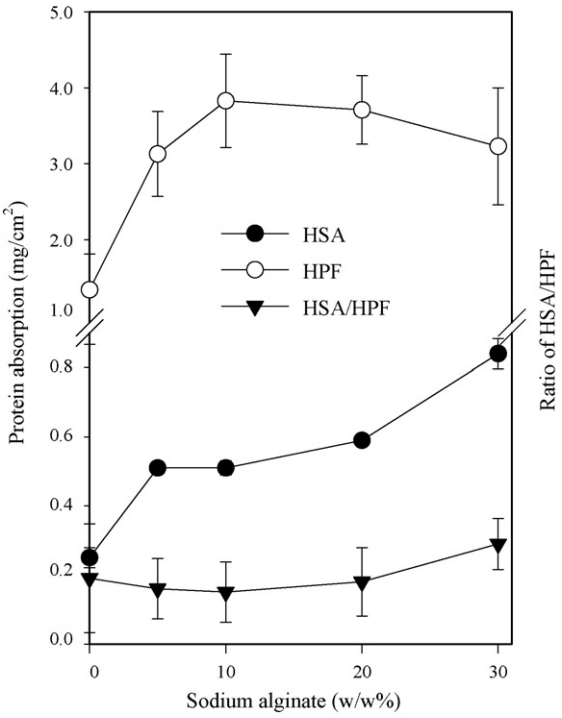

Fig. 7. Effect of SA on protein adsorption (A) and HSA/HPF adsorption ratios (B). Data are expressed as mean ± S.D. (n = 3).

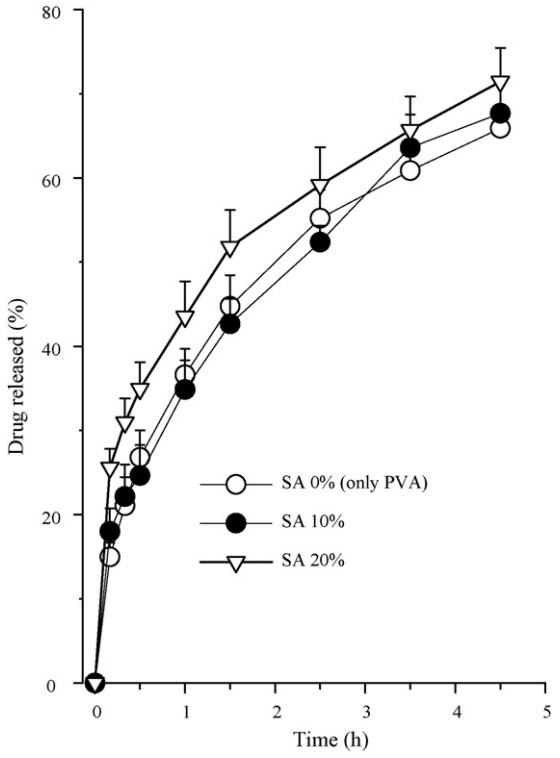

Fig. 8. Effect of SA on the dissolution of nitrofurazone. Data are expressed as mean ± S.D. (n = 3).

to be due to decreased crosslink density (Rosiak et al., 2001). The Young's modulus of the hydrogels was determined and the results are shown in Fig. 4B. From the Young's modulus curve, it can be seen that the more elastic hydrogels were obtained with an increment in SA proportions (Ravi et al., 2003). Our results suggested that the crosslink between PVA and SA was not strong compared to the crosslink between PVA itself. Thus, whereas PVA was hard and close to solid, PVA/SA mixed hydrogels displayed more flexibility and elasticity.

Fig. 5(A) shows almost similar pattern of DSC thermograms for a series of PVA/SA hydrogels. The T_m of reacting hydrogels increased with increasing SA proportion. It was higher than that

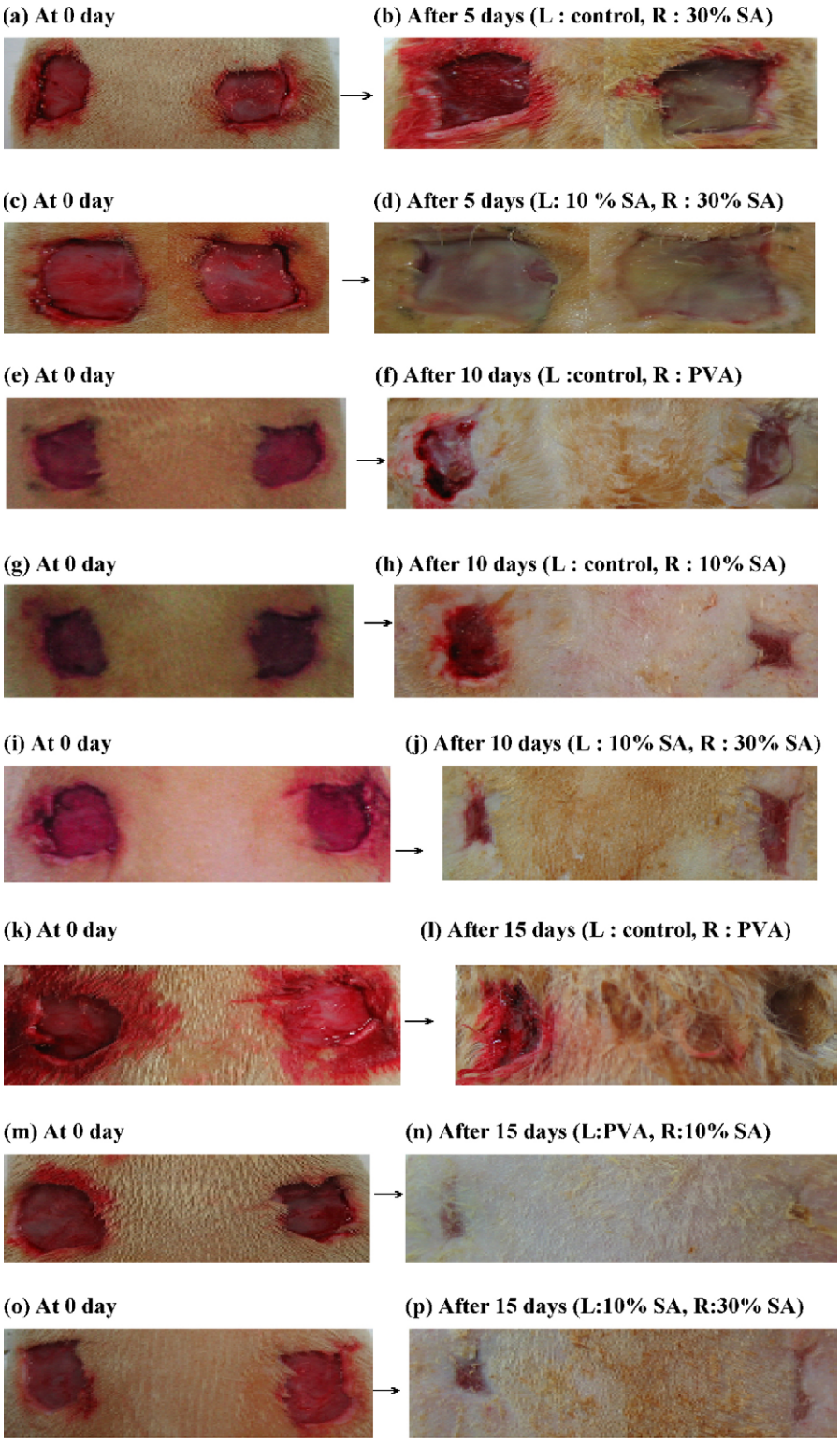

Fig. 9. Representative photographs of wound spot at 0, 5, 10 and 15 days.

of PVA alone. Fig. 5(B) and Fig. 6 showed TGA curve and derivative thermo gravimetric (DTG) peak temperature (T_p) of PVA/SA hydrogels, respectively. They were higher than those of PVA, indicating that SA can improve the thermal stability of PVA, because the carboxyl and hydroxyl groups of SA formed hydrogen bond crosslinking with hydroxyl groups of PVA (Lin et al., 2006). This limited the chain movement during thermal treatment.

3.2. In vitro experiments

When the material was placed in contact with blood, the first event to occur was the adsorption of proteins onto the surface followed by the platelet adhesion and activation (Coleman et al., 1982; Ikada et al., 1982), which could be dictated by type and the amount of blood proteins adsorbed at the biomaterial and blood interface. So, if the albumin adsorption on the synthetic surfaces can inhibit platelet activation it does not promote clot formation whereas the fibrinogen's adsorption is known to accelerate platelet adhesion and activation. Previous studies proposed that the albumin/fibrinogen adsorption ratio is important when assessing the adhesion of platelets onto artificial surfaces (Coleman et al., 1982). They concluded that the higher is the ratio; the lower is the number of adhering platelets. Fig. 7 shows the albumin (HSA) and fibrinogen (HPF) adsorptions onto the PVA/SA membranes and the albumin/fibrinogen adsorption ratio (HAS/HPF). The albumin/fibrinogen adsorption ratio is important when assessing the adhesion of platelets to artificial surfaces. Furthermore, the higher was the albumin/fibrinogen adsorption ratio, the lower was the number of adhering platelets (Dion et al., 1993). As shown in Fig. 7, both the adsorption of HSA and HPF increased as the amounts of SA in the PVA hydrogels increased, even if there were no significance in the HPF values. Furthermore, among the albumin/fibrinogen adsorption ratios tested, the hydrogel with 30% showed highest, indicating that SA affected the adhesion of platelets to artificial surfaces.

To evaluate whether sodium alginate affected the release rates of nitrofurazone, we performed the release studies on various nitrofurazone-loaded hydrogels. The release profiles of nitrofurazone in three nitrofurazone-loaded hydrogels are illustrated in Fig. 8. The release rate of nitrofurazone in the hydrogel with 10% SA hardly changed compared to hydrogel with only PVA in distilled water. Furthermore, the release rate of nitrofurazone in the hydrogel with 20% SA a little increased compared to hydrogel with only PVA but there was no significant difference. Therefore, SA hardly affected the nitrofurazone release behavior from hydrogels.

3.3. In vivo wound healing test and histopathology

Each wound was observed for a period of 5, 10 and 15 days postoperation (Fig. 9). All rats survived throughout the postoperative period until sacrifice. The healing process for each wound treated by dressing application progressed satisfactorily without

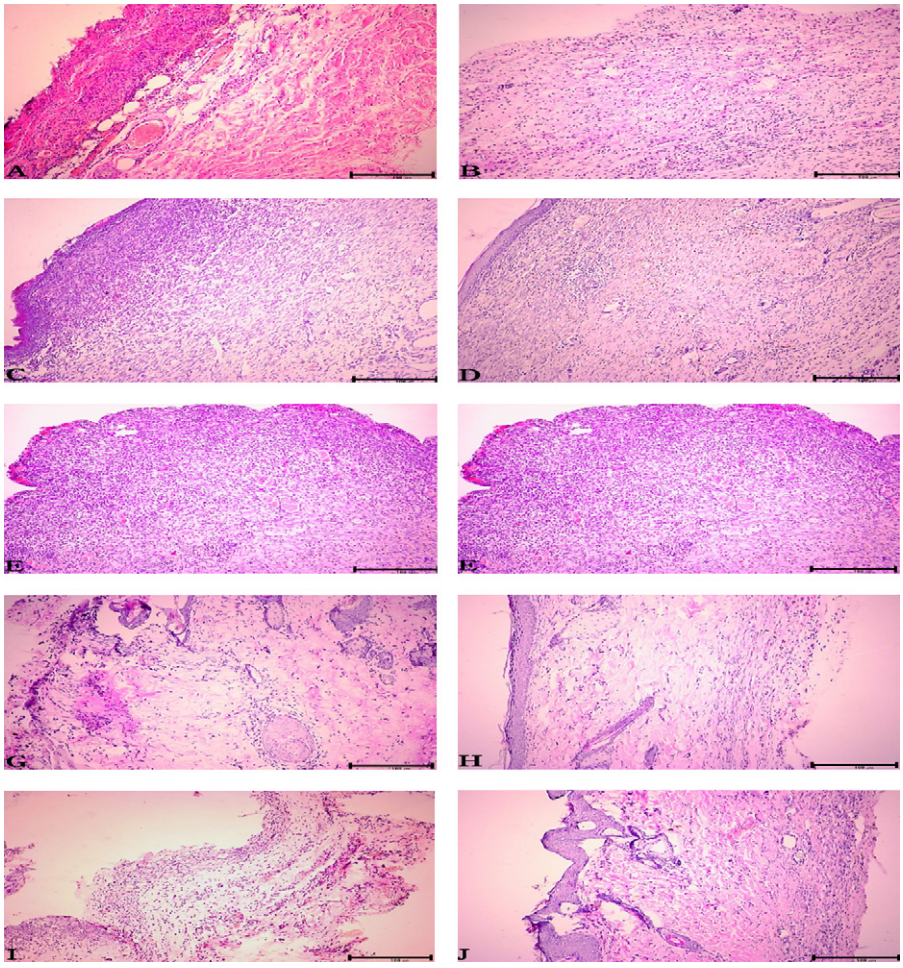

Fig. 10. Histopathological studies. Left photograph (a, c, e, g and i) and right photograph (b, d, f, h, j) were at 10 and 15 days after injury. A and B: control; C and D: PVA; E and F: 10% SA; G and H: 20% SA; I and J: 30% SA. Bar = 40 μm 100×.

any apparent complications. There were no evidences of necrosis. At 5 days postoperatively (b, d), little discrete inflammation was observed. There was no evidence of infection or contraction of the wound, whereas skin was hemorrhagic for some control samples and also scab was present on the wound spot. There was no size reduction in wound defect area of every sample. At 10 days postoperatively (f, h, j), subcutaneous aspects appeared in test and control wounds. Control group shows hemorrhage by second damage while removing the gauze. Crosslinked hydrogel keep moist environment. Moist environment prevent second damage when change dressing. In addition, accelerate epithelialization and keratinocytes migration occurs more easily (Winter, 1962). In size reduction, it shows that better wound healing ability proportionate to the amount of SA. At 15 days postoperatively (l, n, p), majority of the wounds appeared to be healed. Exhibited an external cicatrisation but almost completely sealed.

Healing pattern of wounds was studied by the histology of control, SA 0, 10, 20 and 30% at 5, 10 and 15 days postoperatively (Fig. 10) (Balakrishnan et al., 2005; Knighton et al., 1981). At 5 days of post-wounding, inflammation was observed in every wound in this period. Inflammatory tissue was observed in dermis. Lymphocyte, monocyte and fibroblast were seen. A little hemorrhage was seen too. Inflammatory phase is essential to healing. PVA and PVA/SA groups show mild positive effect compared with control. And there were little difference between PVA only and PVA/SA groups. At 10 days, group of this period is best to compare healing pattern between each sample. PVA/SA wounds appeared reduced in size with new epithelium noted at the edge of the defect. It shows apparent difference between groups. It still showed neutrophils. But in PVA/SA samples less neutrophils were observed compared to PVA only. Furthermore, it had better cell arrangement to healing. At 15 days, in PVA/SA wounds, the defected area almost disappeared and filled with fibro- proliferative tissue. The surface of the defect was covered with new epithelium. Inflammatory cells were absent. However, control wounds didn't completely covered with new epithelium and still showed inflammatory cell. The 30% SA group showed best healing ability. Whole epidermis regenerated (Roh et al., 2006).

PVA is the biodegradable and nontoxic polymer. The natural polymer, SA, is cheap and very useful for wound dressing. As PVA and SA had high aqueous solubility, they needed crosslinking to be applied to the human body as a wound dressing system. Most of the crosslinking method use chemical agent having toxic ketone group. PVA hydrogels produced by freezing–thawing method form a physically crosslinked polymeric chains containing uncrosslinked polymer and water. These gels are non-toxic, non-carcinogenic, and have good biocompatibility and desirable physical properties. In this work, crosslinked hydrogels were prepared at the various proportion of SA to PVA, 0, 5, 10, 20 and 30%. Increasing the concentration of SA, decreased the gelation%, maximum strength and break elongation; and increased the swelling ability, elasticity and thermal stability of hydrogel film. However, SA had insignificant effect on nitrofurazone release behavior. Similarly, the amounts of proteins adsorbed on hydrogel were increased with increasing sodium alginate ratio, indicating the reduced blood compatibility. In vivo experiments showed that the addition of SA is expected to improve utility as wound dressing mildly.

4. Conclusion

In conclusion, the nitrofurazone-loaded wound dressing developed using freezing–thawing method with PVP and SA gave more swellable, flexible and elastic compared to that with only PVA. Furthermore, it showed positive healing effect similar to that with only PVA. Thus, it could be used as a potential wound dressing form with better forming and well healing effect of nitrofurazone.

On the other hands, in this study, the effective healing effect was not known to be due to by PSA-SA mixed polymer or nitrofurazone in wound dressing system. So, for the development of nitrofurazone-loaded wound dressing, the further study on antibacterial activity will be carried out compared to the wound dressing without drug.

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