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International Journal of Pharmaceutics 341 (2007) 35–43
INTERNATIONAL JOURNAL OF PHARMACEUTICS
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Evaluation of semi-interpenetrating polymer networks composed of chitosan and poloxamer for wound dressing application

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Received 30 November 2006; received in revised form 22 March 2007; accepted 28 March 2007
Available online 1 April 2007

Abstract

We have elsewhere reported the work on the preparation of semi-interpenetrating polymer networks (SIPNs) composed of chitosan (CS) and poloxamer to improve the mechanical strength of CS sponge. This study focuses on evaluation of the CS/poloxamer SIPNs to intend for wound dressing application and the efficacy of dehydroepiandrosterone (DHEA)-loaded CS/poloxamer SIPNs in the wound model studies. The properties required for ideal wound dressing, such as equilibrium water content (EWC), water absorption (A_w), water vapor transmission rate (WVTR), and evaporative water loss, were examined. The CS/poloxamer SIPNs were found to have a water content of 90% of their weight which could prevent the wound bed from accumulation of exudates and also have excellent water adsorption. The WVTR of CS/poloxamer SIPNs was found to be $2508.2 \pm 65.7\ g\ m^{-2}\ day^{-1}$, indicating that the SIPNs can maintain a moist environment over wound bed in moderate to heavily exuding wound which enhances epithelial cell migration during the healing process. Also, the CS/poloxamer SIPNs *in vitro* assessment showed proper biodegradation and low cytotoxicity for wound dressing application. The wound healing efficacy of CS/poloxamer SIPNs as a wound dressing was evaluated on experimental full thickness wounds in a mouse model. It was found that the wounds covered with CS/poloxamer SIPNs or DHEA-loaded CS/poloxamer SIPNs were completely filled with new epithelium without any significant adverse reactions after 3 weeks. The results thus indicate that CS/poloxamer SIPNs could be employed in the future as potential wound dressing materials.

Keywords: Chitosan; Poloxamer; Semi-interpenetrating polymer networks; Wound dressing; Dehydroepiandrosterone

1. Introduction

During the past two decades, there has been a need to looking for a novel method for the treatment of burn wounds or extensive skin loss. Generally, wound dressing is used to protect a skin defect from infections and dehydration in the intervening period between hospitalization and grafting. Various natural and synthetic polymers with good biocompatibility have been used in order to develop wound dressing materials. These materials include synthetic polymers such as polyurethane (Taylor et al., 2005), poly(ethylene glycol) (Kim et al., 2000), polycaprolactone (Schwope et al., 1977), poly(lactide-*co*-glycolide) (Lee et al., 2003), polyacrylonitrile (Groth et al., 2002), poly(amino acid) (Hwang and Stupp, 2000), silicone rubber (Crowder and Gooding, 1997), natural polymers such as alginate (Hashimoto et al., 2004), chitosan (Muzzarelli et al., 2005), gelatin (Choi et al., 1999) and collagen (Ma et al., 2003), etc. The ideal wound dressing should have the following properties: (1) provide a moisturized wound healing environment, (2) provide thermal insulation, (3) be removable without causing trauma to the wound, (4) remove drainage and debris, (5) be free from particulate and toxic product, and (6) promote tissue reconstruction processes (Wittaya-areekul and Prahsarn, 2006).

Chitosan (CS), a biopolymer comprising glucosamine and *N*-acetylglucosamine, is an *N*-deacetylated product of chitin and

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doi:10.1016/j.ijpharm.2007.03.042

one of the most abundant polysaccharides in nature. CS has many advantages for wound dressing, namely biocompatibility, biodegradability (Tomihada and Ikada, 1997), hemostatic activity (Abhay, 1998), anti-infection and wound healing acceleration properties (Ueno et al., 2001; Suzuki et al., 1994). For these reasons, CS has been one of the important biomaterials for wound management in recent years. However, the poor mechanical strength of CS itself limits its further use in wound management (Wu et al., 2004). Therefore, an addition of other synthetic polymers is necessary to achieve film or sponge with improved strength and elasticity.

In previous study, we already reported the possibility of semi-interpenetrating polymer networks (SIPNs) composed of chitosan and poloxamer macromer as a wound dressing (Kim et al., 2006). We introduced SIPNs system to provide the mechanical strength required for wound dressing application. The advantages of this system are enhancement of mechanical strength of the network and increase in the compatibility of the polymer blends, which exhibit favorable properties of phase-separated materials (Sperling, 1981). Poloxamer macromer, having acrylated-terminated PEOs, was chosen as a blending material and cross-linked in the presence of CS to form the SIPNs. Poloxamer hydrogels have also been used in a variety of biomedical fields such as medical, pharmaceutical, and cosmetic systems (Ruel-Gariepy and Leroux, 2004). Furthermore, Nalbandian et al. reported that the non-ionic poloxamer significantly enhanced the rate of wound healing through yet unknown mechanism, possibly by stimulating the epithelial growth factor (EGF) (Nalbandian et al., 1987).

The present study was designed to evaluate CS/poloxamer SIPNs as an ideal wound dressing by the water uptake, water vapor permeation, evaporative water loss, *in vitro* biodegradation and cytotoxicity studies in the cultured keratinocytes and fibroblasts. We further examined wound healing in a mouse skin defect model.

2. Materials and methods

2.1. Materials

Chitosan (MW: 300,000; degree of deacetylation: 81%) was kindly provided by Jakwang (Ansung, Korea). Poloxamer 407 (MW:12,600) was provided BASF Korea Inc. (Seoul, Korea) and was used without further purification. Acryloyl chloride, triethylamine, 2,2-dimethoxy-2-phenyl acetophenone, and *N*-vinyl pyrrolidone were purchased from Aldrich (Milwaukee, WI, USA) and used without further purification. Lysozyme from chicken egg white and dehydroepiandrosterone (DHEA) were a commercial product of Sigma Co. and used without further purification. All other chemicals were of extra-pure reagent grade and used as received.

2.2. Synthesis of poloxamer macromer

Poloxamer macromer was synthesized according to the method by Yoo et al. (2004). Briefly, poloxamer 407 (25.2 g; 2 mmol) was dissolved in 75 ml benzene in a round-bottom flask, into which 0.59 ml (4.23 mmol) triethylamine and 0.34 ml (4.18 mmol) acryloyl chloride were added. After stirring for 3 h at 80 °C, the reaction mixture was filtered to remove triethylamine-hydrochloride, and the macromer was obtained by dropping the filtrate into an excess of *n*-hexane. Finally, the precipitated poloxamer macromer was dried at 40 °C under reduced pressure for 24 h.

2.3. Preparation of CS/poloxamer SIPNs

The procedure of preparation of CS/poloxamer SIPNs is the same as our previous study (Kim et al., 2006). Briefly, 25 μl radical initiator solution (100 mg of 2,2-dimethoxy-2-phenyl acetophenone dissolved in 1 ml *N*-vinyl pyrrolidone) was added to 1 wt.% aqueous solution of chitosan, and 1, 2 or 3 wt.% poloxamer macromer. The DHEA (2 wt.% of polymer weight) dissolved in 200 μl of 99% ethanol was also added to the mixture and homogenized by stirring. Then, the mixture loaded DHEA was irradiated for 5 min using a low-intensity LWUV lamp (Toshiba Chemical Lamp FL 20 LB; wave range, 300–400 nm; maximum intensity, 360 nm) and freeze-dried to obtain sponge-type materials. The prepared SIPNs were then neutralized with 2 wt.% aqueous NaOH solution for 30 min and subsequently washed with water to remove the remaining NaOH.

2.4. Equilibrium water content and water absorption

The CS/poloxamer SIPNs were incubated in PBS (pH 7.4) at 37 °C for 24 h, and swelling continued to reach constant weight of the sample. Before weighing the sample, surface water was removed with filter paper. The swelled sample was then slowly dried to the constant weight.

The equilibrium water content (EWC) and the water absorption (A_w) were calculated by the following equation:

$$\mathrm{EWC}\,(\%) = \frac{W_s - W_d}{W_s} \times 100$$

where W_s and W_d are the weights of swollen state and dried state, respectively:

$$A_w\,(\%) = \frac{W_s - W_i}{W_i} \times 100$$

where A_w is water absorption and W_i is the weight of initial sample (before being immersed in water).

2.5. Water vapor transmission rate

The water vapor transmission rate (WVTR) across the CS/poloxamer SIPNs was determined as stipulated by ASTM Standard (2000). The CS/poloxamer SIPNs were mounted on the mouth of cylindrical plastic cups (28 mm diameter) containing 10 ml water with negligible water vapor transmission, and placed in an oven at 35 °C for 24 h. The weight loss versus time plot was weighed at regular intervals of time. The WVTR was also calculated by using the following formula:

$$\mathrm{WVTR} = \frac{W_i - W_t}{A} \times 10^6\ \mathrm{g/m^2\,day^{-1}}$$

where WVTR is expressed in g/m^2 h, A the area of bottle mouth (mm^2), W_i and W_t are the weight of bottle before and after placed in oven, respectively.

2.6. Evaporative water loss

The samples were kept at 37 °C and 35% relative humidity. After regular intervals of time, the weight of CS/poloxamer SIPNs was measured. Water loss was estimated by the following equation:

$$\text{water loss}\,(\%) = \frac{W_t}{W_0} \times 100$$

where W_0 and W_t are the initial weight after 24 h immersion time and weight after time 't', respectively.

2.7. In vitro biodegradation

The samples with a thickness of 1–2 mm were cut into small pieces (1 cm × 1 cm) and were immersed in 5 ml phosphate-buffered solution (PBS, pH 7.4) at 37 °C containing 1.5 mg/ml lysozyme (hen egg-white, Sigma-Aldrich, Oakville, Canada). The concentration of lysozyme was chosen to correspond to the concentration in human serum (Brouwer et al., 1984). The lysozyme solution was daily refreshed to ensure continuous enzyme activity (Masuda et al., 2001). After 1, 4, 7, 10 and 14 days, samples were removed from the medium, rinsed with distilled water, freeze-dried and weighed. The extent of in vitro degradation was expressed as percentage of weight loss of the dried samples after lysozyme treatment.

2.8. Cell culture

For the cytotoxicity test, the fibroblast cell line NIH 3T3 (ATCC, USA) and the human keratinocyte cell line HaCaT (ATCC, USA) were grown as a monolayer in DMEM (Hyclone Lab. Inc., USA), supplemented with 10% heat-activated FBS (Hyclone Lab. Inc., USA), 100 IU/ml penicillin and 100 μg/ml streptomycin in a humidified incubator with 5% CO_2 in air at 37 °C. Cells were plated at density of 10×10^4 in 12-well plates treated with CS and CS/poloxamer SIPNs and were incubated for 24 h.

2.9. Lactate dehydrogenase release assay

The lactate dehydrogenase (LDH) activity assay was performed with the CytoTox96® Non-Radioactive Cytotoxicity Assay kit (Promega, Madison, WI, USA), which quantitatively measures the activity of LDH, a stable cytosolic enzyme that is released upon cell lysis (Technical Bulletin No. 163, Promega). LDH in culture supernatants was measured with a 30-min coupled enzymatic assay, which resulted in the conversion of a tetrazolium salt into a red formazan product. The amount of formed color was proportional to the degree of damage to the cell membranes. Absorbance data was collected using a microplate reader at 490 nm. Control experiments were performed with 0.1% (w/v) Triton X-100 and set as 100% cytotoxicity. LDH release was calculated by the following equation:

$$\text{cell viability}\,(\%) = \left[1 - \left(\frac{[A]_{\text{sample}} - [A]_{\text{medium}}}{[A]_{100\%} - [A]_{\text{medium}}}\right)\right] \times 100$$

where $[A]_{\text{sample}}$, $[A]_{\text{medium}}$ and $[A]_{100\%}$ denote the absorbance of the sample, medium control and Triton X-100 control, respectively. All experiments were run in triplicate.

2.10. Preparation of thermal injury

BALB/c mice, ranging in age from 6 to 8 weeks, were obtained from Korea Advanced Institute of Science and Technology (KAIST, Taejon, Korea). A group of four female mice was used in each experiment. Briefly, after induction of general anesthesia with ketamine and xylazine to achieve deep anesthesia, BALB/c mice were shaved over a dorsal area using clippers and exposed for 30 s to the round iron metal block (1.3 cm in diameter; Thermolyne Inc., USA) at 95 °C with 20 g/cm^2 pressure. Dorsal full-thickness burn was given in a standardized manner in accordance with the Guide for the Care and Use of Laboratory Animals (DHEW publication no. NIH 78–23, revised 1985). Each burn wound was covered with wound dressing (size: 2 cm × 2 cm and thickness: 3 mm) and then fixed with gauze and elastic tape. Three days after coverage, the burn wound was surgically excised and the wound was covered with new wound dressing and thereafter changed every week.

2.11. Histological observation

Three weeks after treatment with various dressings, the mice were sacrificed and the wound tissues were obtained, and fixed in 10% formaldehyde. Fixed specimens were sectioned and stained with hematoxylin–eosin for histological evaluation.

3. Results and discussion

3.1. Synthesis of poloxamer macromer and poloxamer networks

The scheme of poloxamer macromer is illustrated in Fig. 1(a). As described in our previous study (Kim et al., 2006), the poloxamer macromer was obtained through the reaction between the terminal hydroxyl groups of poloxamer and acryloyl chloride. The synthesis of poloxamer macromer was clarified by ^{1}H NMR spectroscopy. The substitution degree of poloxamer macromer calculated from NMR measurement was 87.2 mol%. Subsequently, unreacted poloxamer was removed by washing with NaOH aqueous solution and distilled waster after SIPNs formation because poloxamer macromer was cross-linked by UV treatment using photoinitiator. Fig. 1(b) shows the poloxamer networks prepared through UV irradiation of poloxamer macromer.

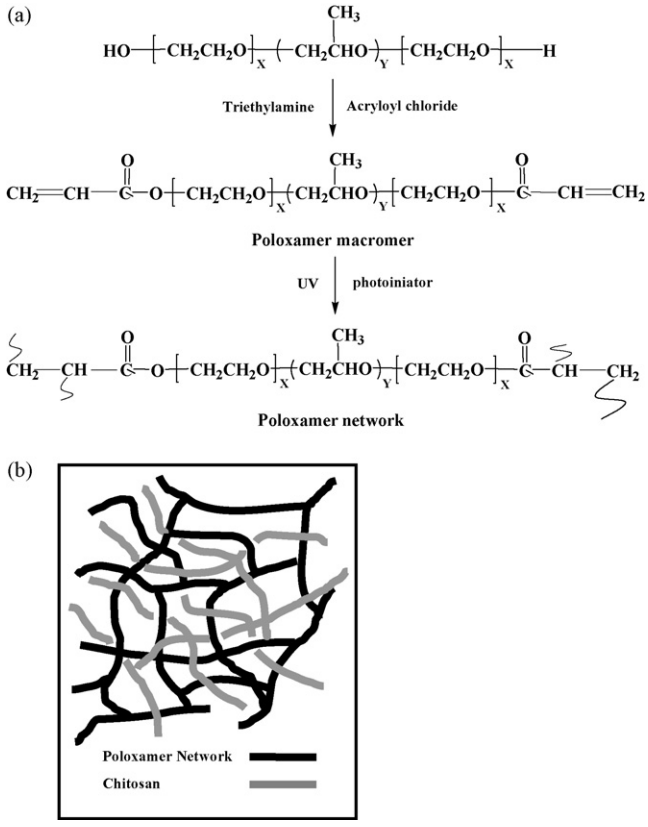

Fig. 1. Synthesis scheme of poloxamer macromer and poloxamer networks from poloxamer macromer (a), and structure of CS/poloxamer SIPNs (b).

3.2. Equilibrium water content and water absorption

The equilibrium water content (EWC) and water absorption (A_w) of wound dressing are important for quick absorption of exudates. As shown in Table 1, the EWC of CS/poloxamer SIPNs showed water uptake ability similar to that of CS itself or more. The A_w of CS/poloxamer SIPNs also showed a tendency like the EWC. These observations may be explained as follows. Poloxamer network is a type of hydrogel that swells in water, thereby retaining a significant amount of water within its structure. In addition, CS is a cationic polysaccharide with water-binding capacity and has abundant hydroxyl groups necessary for hydrogen interaction with water. On the other hand, the EWC and A_w of CS/poloxamer SIPNs decreased with increasing poloxamer content due to the increased cross-linking density of poloxamer in the SIPNs. The less water adsorption ability would be also attributed to the decreased pore diameter of CS/poloxamer SIPNs, resulted in the decrease of void to capillary adsorbed water. In previous study, we confirmed that the pore diameter decreased with increasing of poloxamer content in the CS/poloxamer SIPNs (Kim et al., 2006). These results reveal that the CS/poloxamer SIPNs with a proper concentration of poloxamer could have potential to prevent wound from accumulation of fluid by the adsorption of exudate.

3.3. Water vapor transmission rate

The most difficult problem in treating a burned was the fact that the victim may have lost most of its body liquid due to evaporation and exudation. These will affect the decrease of body temperature and accelerate the rate of metabolism (Peppas, 1987). Therefore, the wound dressing must avoid or at least reduce the body liquid lost, i.e. by controlling absorption and transmission as well as by maintaining the high humidity in the wound area, in order to accelerate the formation of granule and epithelialization process. On the other hand, if the WVTR is low, then accumulation of exudates will occur, which may cause the deceleration of healing process and opens up the risk of bacterial growth. Lamke et al. (1977) reported the evaporative water loss for normal skin as $204 \pm 12\ g\ m^{-2}\ day^{-1}$ and that for injured skin can range from $279 \pm 26\ g\ m^{-2}\ day^{-1}$ for a first degree burn to $5138 \pm 202\ g\ m^{-2}\ day^{-1}$ for a granulating wound. The water vapor permeability 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 $g\ m^{-2}\ day^{-1}$ would provide adequate level of moisture without risk in wound dehydration (Queen et al., 1987). As shown in Table 1, the WVTR of samples covers the ideal range to maintain a proper fluid balance on the wound bed, which can facilitate cellular migration and enhance epithelialization. The WVTR of CS/poloxamer SIPNs also decreased with increasing poloxamer content due to the increased cross-linking density of poloxamer in SIPNs.

3.4. Evaporative water loss

The water loss from the CS/poloxamer SIPNs on exposure to the air was evaluated to examine its behavior when used as a dressing over a dry wound. As shown in Fig. 2, the loss of water was approximately 10% after 1 h and within 6 h it increased up to 90%. After 6 h, the loss of water became negligible and sample retained about 5–10% of water. It is clear from the assessment of evaporative water loss that the material will lose its water content when exposed to air under dry conditions during short periods. Therefore, these dressings may be more beneficial to wounds

Table 1
The water content, water absorption and water vapor transmission rate of different composition of CS/poloxamer SIPNs

Composition [CS/poloxamer (wt%)]	Water content (EWC) (%)	Water absorption (%) in 24 h immersion time	WVTR ($g\ m^{-2}\ day^{-1}$)
CS	95.9 ± 0.8	2410.7 ± 463.8	2212 ± 108
CS/poloxamer (1/1) SIPNs	96.2 ± 0.1	2508.2 ± 65.7	2100 ± 36
CS/poloxamer (1/2) SIPNs	96.0 ± 0.3	2420.6 ± 164.0	2011 ± 86
CS/poloxamer (1/3) SIPNs	94.5 ± 0.4	1730.3 ± 127.9	1938 ± 43

Mean ± S.D. ($n = 5$).

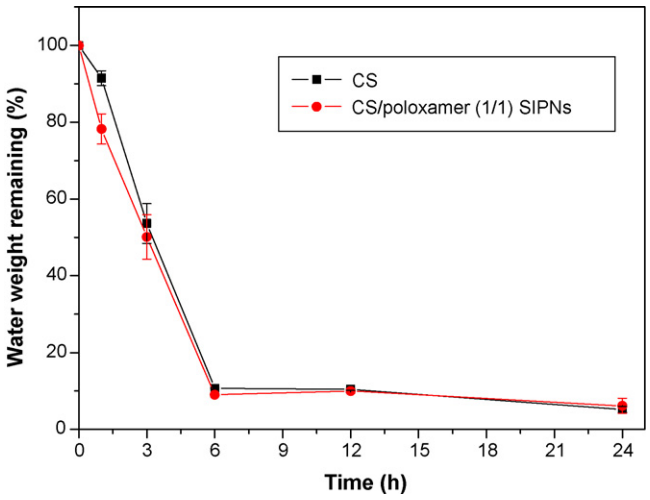

Fig. 2. Evaporative water loss from CS and CS/poloxamer (1/1) SIPNs. Bars represent mean ± S.D. For $n = 5$.

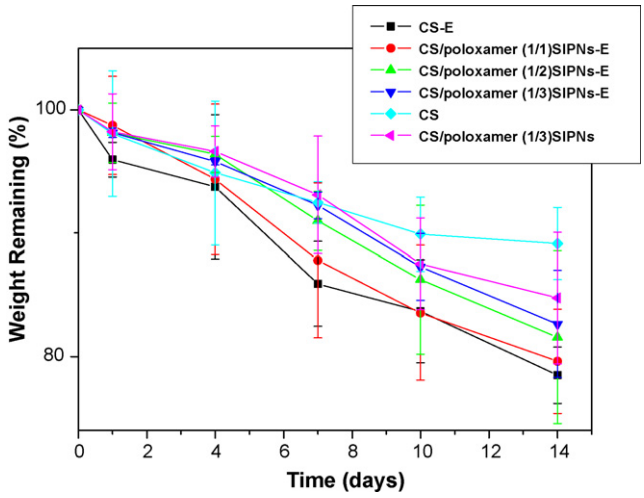

Fig. 3. Weight remaining of the CS, CS/poloxamer (1/1) SIPNs, CS/poloxamer (1/2) SIPNs, and CS/poloxamer (1/3) SIPNs against time. Bars represent mean ± S.D. For $n = 5$.

with more exudates in early-stage wound. That is, extents of the water loss will enable the CS/poloxamer SIPNs to take up quickly exudates and oedema fluid from the wound into the dressing by an active upward-directed process when used in early-stage exuding wounds.

3.5. *In vitro* biodegradation

Fundamental information regarding the enzymatic biodegradation of CS/poloxamer SIPNs should be required for the *in vivo* wound dressing applications. CS is degradable *in vitro* at a slow rate. However, the degradation in presence of lysozyme can be accelerated. In human serum, CS is mainly depolymerized enzymatically by lysozyme, and not by other enzymes or other depolymerization mechanisms. The enzyme biodegrades the polysaccharide by hydrolyzing the glycosidic bonds present in the chemical structure (Pangburn et al., 1982). To mimic the *in vivo* degradation performance, the degradation of CS alone and CS/poloxamer SIPNs prepared with different poloxamer contents was examined at pH 7.4 in phosphate buffer saline (PBS) with lysozyme. We compared the degradation of samples *in vitro* between with and without lysozyme. As shown in Fig. 3, the samples with lysozyme showed significant enzymatic degradation compared with the ones without lysozyme. Also, the weight loss of CS/poloxamer SIPNs decreases with increasing poloxamer content in the SIPNs. Indeed, this can be clearly explained by noticing the hydration properties of CS/poloxamer SIPNs. Those having lower poloxamer content have better accessibility to water and thus prone to more degradation. Contrastively, at higher poloxamer content, the increased crosslinking density in SIPNs may become the dominant factor, which will lead to lower degradation. Moreover, the rather high crosslinking brings about stereo-hindrance of the enzyme for approaching the glycosidic bonds. On the other hand, in PBS without lysozyme, the weight loss of CS alone was lower than that of CS/poloxamer SIPNs. That is, the hydrolytic degradability of CS/poloxamer SIPNs is larger compared with that of CS alone because the poloxamer in CS/poloxamer SIPNs has ester linkage prone to hydrolyze.

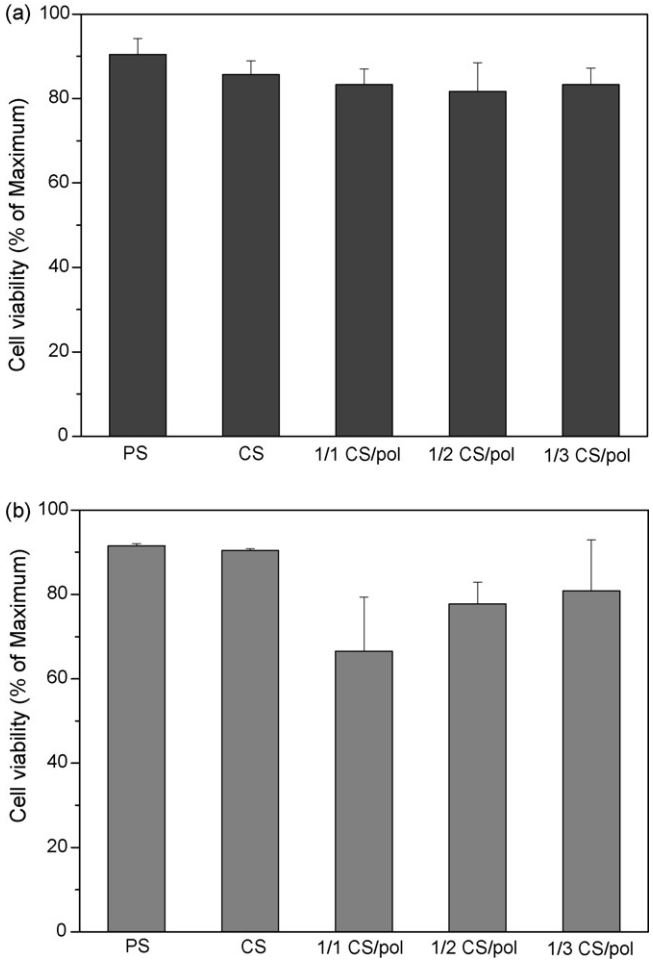

Fig. 4. Cytotoxicities of the CS, CS/poloxamer (1/1) SIPNs, CS/poloxamer (1/2) SIPNs, and CS/poloxamer (1/3) SIPNs on HaCaT keratinocytes (a) and NIH 3T3 fibroblasts (b) by LDH assay. Bars represent mean ± S.D. For $n = 5$.

3.6. Cytotoxicity

Generally, the *in vitro* cytotoxicity of biomaterials has been evaluated by determining the cell viability. The *in vitro* cytotoxicity test is based on the concept that toxic chemicals affect the basic functions of cells. Such functions are common to all cells, and hence the toxicity can be measured by assessing cellular damage. LDH assay was frequently used for this purpose. LDH is a stable enzyme present in the cytosol that is released upon cell lysis. This assay permits the investigation of chemicals that may induce alterations in cell integrity. It was performed to measure the membrane-damaging effects of the materials via the quantification of LDH in the culture media after 24 h. We evaluated the cytotoxicity of keratinocytes and fibroblasts on the CS/poloxamer SIPNs prepared with different poloxamer contents. The cytotoxicities on the CS and CS/poloxamer SIPNs were also compared with that on polystylene (PS) as a control. As shown in Fig. 4, these two types of cells showed an excellent viability on the CS and CS/poloxamer SIPNs within 24 h, compared with on the control. The cell survivals observed on the CS and CS/poloxamer SIPNs are not unexpected because CS has been previously demonstrated as a biocompatible material for various types of cells (Ueno et al., 2001; Risbud et al., 2001; Chupa et al., 2000) and the poloxamer on the SIPNs has also shown to be biocompatible with cells and tissues (Park and Park, 1996). Furthermore, the viabilities of all the cells showed the tendency of increase with increasing poloxamer content on CS/poloxamer SIPNs. This result can be explained by the fact that cell behavior on the SIPNs is attributed to the physical changes resulting from crosslink density variations. Namely, the initial effect of molecular mobility on biocompatibility is independent of morphologic order/disorder and crystallinity (Mirzadeh et al., 2003).

3.7. Wound contraction

Fig. 5 shows photographs of macroscopic appearance of the wound contraction at 3 weeks after wound dressing with gauze as a control group (A), vaseline gauze (B), CS/poloxamer SIPNs (C), and DHEA-loaded CS/poloxamer SIPNs (D). DHEA (2 wt% of polymer weight) as an immune enhancer was used in full-thickness burn wound healing (refer to the part of histological observation for details). As seen in Fig. 5, subcutaneous aspect showed grossly normal for the test samples and there was no evidence of infection, while a superficial skin was still haemorrhagic for control group and also scab was present on the wound bed. The wound dressing with gauze did not effectively elicit wound contraction compared with CS/poloxamer SIPNs

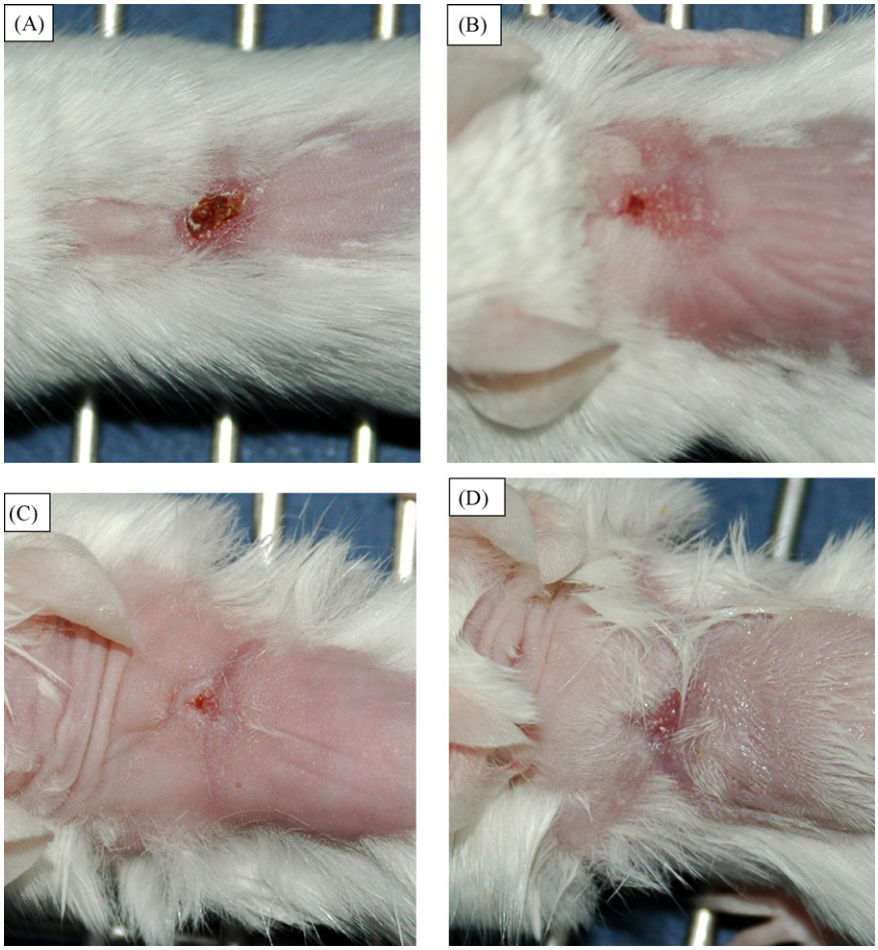

Fig. 5. Photographs of macroscopic appearances of wound contraction treated with gauze (A), vaseline gauze (B), CS/poloxamer SIPNs (C), and DHEA-loaded CS/poloxamer SIPNs wound dressing (D) 3 weeks after application.

and DHEA-loaded CS/poloxamer SIPNs. It has been reported that epithelialization is retarded by the dry scab (Winter and Scales, 1963). The moist wound surface provides an environment that epidermal cells can migrate more easily than a dry scab. On the other hand, complete wound contraction was induced by the DHEA-loaded CS/poloxamer SIPNs wound dressing after 3 weeks. The CS/poloxamer SIPNs and DHEA-loaded CS/poloxamer SIPNs gave rise to almost complete contraction although the wound contraction of the CS/poloxamer SIPNs was not complete.

3.8. Histological observation

To evaluate the quality of the regenerated tissue, we performed hematoxylin–eosin (H&E) staining of the wound-edge tissue on the 3 weeks of post-wounding with changing the respective dressings every week. Generally, the wound healing initiates from inflammatory phase that is a normal and necessary prerequisite for healing (Balakrishnan et al., 2005). During early stage of wound healing, it is difficult to assess whether the inflammatory response is part of normal healing process or due to the effect of material. Fig. 6 shows the histological photographs of the injured surface covered with gauze as a control group (A), vaseline gauze (B), CS/poloxamer SIPNs (C), and DHEA-loaded CS/poloxamer SIPNs (D). In Fig. 6(A), the wound surface was covered by exudate layer which was consisted of the mixture of the fibrin, tissue debris and polymorpho nuclear leukocyte (PMN), and the epidermis was partially restored. Also, the granulation tissue containing the new capillaries, abundant fibroblasts and variable numbers of inflammatory cells are actively formed in the disrupted site due to the discontinuity of the epithelium. The focal irregular area of the epithelium noted for some of the test wounds was found only in the control group. Basal layer of the epidermis was smooth and no epidermal rete pegs were formed. In Fig. 6(B), the continuity of the epithelium was completely restored and some epidermal rete pegs and the desquamation also noted. In addition, the granulation tissue in the dermis developed less than that in the control group. Fig. 6(C) and (D) shows the wound sites treated with CS/poloxamer SIPNs and DHEA-loaded CS/poloxamer SIPNs, respectively. In Fig. 6(C), the epidermal continuity was completely restored and some epidermal rete pegs and desquamation were also noted, however, debris still partially covered the surface of the epidermis and the granulation tissue was noted. Granulation tissue formation is essential for permanent wound closure, since it fills the defects and prepares the way for epithelialization. These findings support that CS/poloxamer SIPNs are able to provide suitable condition for granulation tissue formation. Fig. 6(D) showed that the injured site was restored almost to normalcy. The wound site filled with fibro-proliferative tissue and the granulation tissues were markedly reduced in the dermis. The entire surface of the defect was covered with new epithelium. The epidermal rete pegs were well developed and mature collagen was also present in dermis. Moreover, the hair-germs were found at the stratum basal, although no hair follicle was found in the dermis, and the wound showed some sebaceous gland under the

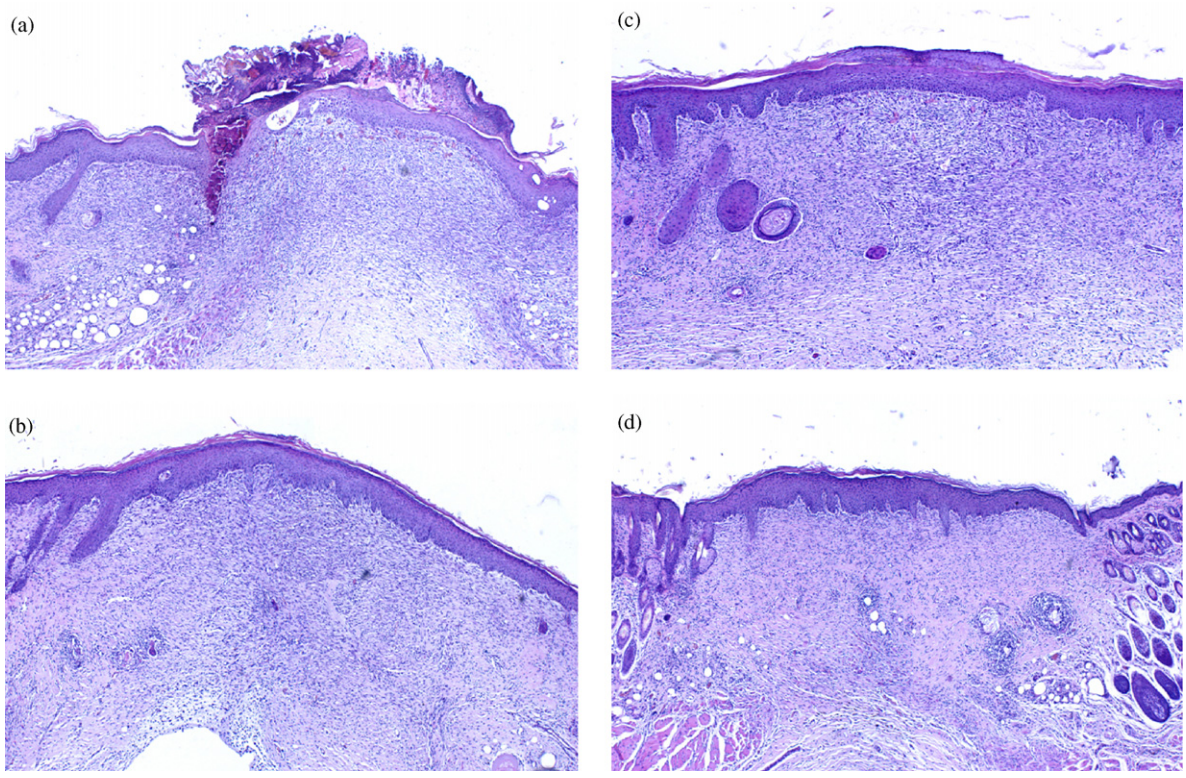

Fig. 6. Histological photographs of skin surface treated with various wound dressings (3 weeks after application): gauze (a); vaseline gauze (b); CS/poloxamer SIPNs (c); and DHEA-loaded CS/poloxamer SIPNs wound dressing (d).

wound surface. These results indicate that CS/poloxamer SIPNs as wound dressing can provide a proper environment for wound healing. On the other hand, the wound healing on the wound site treated with DHEA-loaded CS/poloxamer SIPNs was faster than any other groups. The DHEA is generally known an adrenal androgen in man and other species. One of DHEA's postulated biological roles is to inhibit glucose-6-phosphate dehydrogenase (Marks and Banks, 1960) and act as a potential precursor for other steroid hormones (Meikle et al., 1991). Also, the effects of DHEA on immune and inflammatory reactions were explained by Daynes et al. who suggested that splenocytes from mice treated with exogeneous DHEA or DHEAS showed an increased ability to secrete interleukin-2 (IL-2) but not IL-4 in response to mitogen or antigen (Dayes et al., 1990). They further reported that administration of DHEA to animals given a 20% burns of total body surface prevented the thermal injury-induced depressions in immune competence and progressive ischemia and that the necrosis of the skin following thermal injury was induced by post-burn administration of DHEA (Araneo et al., 1995). Therefore, the wound healing may be much affected by released DHEA from DHEA-loaded CS/poloxamer SIPNs and the DHEA may also prevent not only progressive destruction of the thermally injured skin tissues, but also the systemic change of the immune function after burn. These histological observations under *in vivo* assessment reveal that the CS/poloxamer SIPNs can meet the efficacy requirement and its healing was comparable to that gauze and vaseline gauze. The SIPNs were also comfortable, and did not disturb the formation of cells and new tissue on the skin (Fig. 6).

4. Conclusions

In previous study, we introduced SIPNs system to prepare hydrogels that combine the beneficial properties of both CS and poloxamer. In present study, the CS/poloxamer SIPNs were evaluated for effective application as a wound dressing. The evaluations of their water uptake, water vapor permeation and evaporative water loss showed optimal conditions for maintaining a properly moist environment conducive for wound dressing. Furthermore, the evaluation of CS/poloxamer SIPNs in enzymatic biodegradation provided necessary information for *in vivo* wound dressing application and the SIPNs also showed a low cytotoxicity on the keratinocytes and fibroblasts. *In vivo* test, granulation tissue formation and wound contraction for the CS/poloxamer SIPNs and DHEA-loaded CS/poloxamer SIPNs wound dressing were faster than any other groups. In consequence, these data support that the CS/poloxamer SIPNs can be safely used as good wound dressing systems.

Acknowledgements

This research was supported by a grant (B-2005-10) from Marine Bioprocess Research Center of the Marine Bio 21 Center funded by the Ministry of Maritime Affairs & Fisheries, Republic of Korea. We thank National Instrumentation Center for Environmental Management (NICEM). I.Y. Kim was supported by Brain Korea 21 program.

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