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Mechanical properties and *in vivo* healing evaluation of a novel *Centella asiatica*-loaded hydrocolloid wound dressing

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ABSTRACT

To develop a novel sodium alginate based *Centella asiatica* (CA)-loaded hydrocolloid wound dressing (HCD) providing excellent mechanical properties and improved wound healing, numerous CA-loaded HCDs were prepared with various ingredients using the hot melting method. The effect of sodium alginate, styrene-isoprene-styrene copolymer (SIS) and petroleum hydrocarbon resin (PHR) on the mechanical properties of CA-loaded HCDs was investigated. The effect of disintegrants on swelling and drug release was assessed. Moreover, the *in vivo* wound healing potentials of the selected CA-loaded HCD in various wound models such as abrasion, excision and infection were evaluated in comparison with the commercial product. Polyisobutylene and SIS hardly affected the mechanical properties, but PHR improved the tensile strength and elongation at break. Disintegrants such as croscarmellose sodium, sodium starch glycolate and crospovidone improved the swelling ratio of the CA-loaded HCD. Furthermore, the CA-loaded HCD without croscarmellose sodium poorly released the drug, but that with 2% croscarmellose sodium showed about 27% drug release in 24 h. In particular, the CA-loaded HCD composed of CA/polyisobutylene/SIS/PHR/liquid paraffin/sodium alginate/croscarmellose sodium at the weight ratio of 1/8/25/25/12/27/2 furnished excellent mechanical properties and drug release. As compared with the commercial product, it offered improved healing effects in excision, infection and abrasion type wounds in rats. Thus, this novel CA-loaded HCD could be a potential candidate for the treatment of various wounds.

1. Introduction

Wound healing is a specific process leading to the restoration of injured tissues (Boateng et al., 2008). In general, it consists of five overlapping phases: hemostasis, inflammation, migration, proliferation and maturation (Velnar et al., 2009). Dry wounds are relatively more prone to infection than moist ones. Moist conditions at the wound site stimulate healing and cosmesis, and mitigate pain and infection (Harkins et al., 2014). Several products are available commercially for treating acute and chronic wounds (Abdelrahman and Newton, 2011). Wound dressings shield wounds from the external environment and accelerate various stages of wound healing by creating better healing conditions at the wound site; however, they might stick to a desiccated wound surface and induce trauma upon detachment. An ideal wound dressing must possess the ability to: (1) absorb surplus exudates from the wound surface, (2) maintain high moistness at the wound site, (3) permit gas exchange, (4) provide thermal insulation, (5) be non-allergenic and non-toxic, (6) protect the wound from the invasion of microbes and (7) detach smoothly without causing trauma to the wound (Jayakumar et al., 2011). Conventional wound dressings such as cotton wool, bandages or gauze do not comply with these merits satisfactorily; therefore, they can make wounds vulnerable to infection (Boateng et al., 2008). On the other hand, numerous modern wound dressings based on collagen, polyurethane, silicone or polyacrylates provide

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rapid and painless wound healing by maintaining optimum moisture and protection from infection and irritating exudates at the wound site. However, many of these modern wound dressings have some disadvantages; thus, the quest for an "ideal" wound dressing material is still in progress. Furthermore, hydrocolloid dressings are frequently employed in wound healing. In these dressings, various elastomers, adhesives and hydrophilic polymers are attached to a semipermeable thin sheet to produce a flat, occlusive and adhesive dressing that possesses the property to form a gel upon contact with wound exudates, thus facilitating moist wound healing (Jones et al., 2006). These dressings sufficiently impart the above-mentioned ideal wound dressing properties; however, drug release from hydrocolloids is insufficient owing to the strong linkages of the hydrophobic polymer chain.

The objective of the present research was to develop a novel *Centella asiatica* (CA)-loaded hydrocolloid dressing (HCD) with excellent mechanical properties and improved wound healing using sodium alginate. The numerous CA-loaded HCDs with different weight ratios of ingredients were prepared using the hot melting method, and their mechanical properties, swelling and dissolution were assessed. Additionally, the *in vivo* wound healings of the selected CA-loaded HCDs were evaluated in various wound models such as abrasion, excision and infection wound models in comparison with the commercial product. Here, CA was used as a model drug in order to develop an HCD with antimicrobial activity. CA, a solid drug contains three major components such as asiaticoside, asiatic acid and madecassic acid (Liu et al., 2008). All these major components are effective in treating abnormal scar formation, systemic scleroderma and keloids (Kimura et al., 2008; Lu et al., 2004) by strongly inhibiting the biosynthesis of collagens and acid mucopolysaccharides in carrageenin granulomas (Song et al., 2012). In addition, this drug possesses excellent bacteriostatic and anti-inflammatory activity (Chomnawang et al., 2005; Mamedov, 2005). It have been reported to induce no irritation or damage to skin (Martelli et al., 2000). Sodium alginate was used as the base of the HCD because of its biocompatibility and excellent hydrocolloid film forming property (Balakrishnan et al., 2005; Devi et al., 2012; Jones et al., 2006; Thomas, 2000).

2. Materials and methods

2.1. Materials

C. asiatica was bought from Acetar Bio-tech Co. (St. Louis, MO, USA). Sodium alginate and styrene-isoprene-styrene copolymer (SIS, Quintac®) were purchased from Aldrich Co. (Milwaukee, WI, USA) and Zeon Co. (Tokyo, Japan), respectively. Polyisobutylene (Oppanol® B 15SFN) and crospovidone (Kollidon® CL) were provided by BASF (Ludwigshafen, Germany). Petroleum hydrocarbon resin (PHR, C5 series) and liquid paraffin were procured from Kolon Co. (Kwacheon, South Korea) and Kukdong Co. (Yangsan, South Korea), respectively. Croscarmellose sodium (Primellose®) and sodium starch glycolate (Primojel®) were purchased from Avebe (Veendam, The Netherlands). A commercial product (Duoderm™; wound dressing form) was purchased from Convatec Korea Co. (Seoul, South Korea). The backing layer and the release liner were kindly supplied by Young Chemical Co. (Yangsan, South Korea). All other chemicals were used without any further purification.

2.2. Animals

The *in vivo* wound healing properties of hydrocolloids were assessed in male Sprague-Dawley rats (250–280 g, 6–7 weeks old) obtained from Nara Biotech (Seoul, South Korea). Moreover, the diabetic rats (blood glucose >250 mg/dl) were used in the abrasion and excision wound models (Peppa et al., 2003). Streptozotocin (Sigma–Aldrich, St. Louis, MO, USA) in saline-sodium citrate buffer (pH 4.5) was intravenously injected to each rat at a dose of 60 mg/kg in order to induce artificial diabetes. After 5 days of injection, blood was sampled from the tail vein of each rat and the glucose titer was measured using a blood glucose meter (Accuchek®; Roche Diagnotics Korea Co., Seoul, South Korea). The procedures for the animal studies were performed according to the NIH Policy and Animal Welfare Act under the endorsement of the Institutional Animal Care and Use Committee (IACUC) of the Dong-A research center.

2.3. Preparation of CA-loaded HCDs

All the CA-loaded HCDs were prepared by the hot melting method. First of all, SIS was melted at 170 °C. Then, the melt was cooled to 120 °C and mixed with polyisobutylene using a mechanical stirrer at 300 rpm for 40 min. An optically clear mixture was obtained, which was further cooled to 100 °C. Subsequently, the drug (*C. asiatica*), sodium alginate, liquid paraffin and PHR were added to it. The whole mixture was thoroughly mixed using a mechanical stirrer at 300 rpm for 20 min. The final hydrocolloid matrix (Fig. 1) was degassed for 10 min and poured onto a backing film using a hot melt coater (HLC-101, ChemInstruments; Mentor, OH, USA). The instrument was set at a temperature of 100 °C and HCD thickness of 0.5 mm. Then, the release liner was added using a benchtop laboratory laminator (LL-100, ChemInstruments; Mentor, OH, USA) with an air pressure of 20 psi.

2.4. Determination of mechanical properties

2.4.1. Tensile strength and elongation at break

A texture analyzer (TA.XT2, Stable Micro Systems, Haslemere, Surrey, UK) was employed for determining the elongation at break and tensile strength of the square-shaped HCD samples (2 mm × 2 mm), according to ASTM D882 (Sabetzadeh et al., 2015). The tensile strength was determined by gradually increasing the weight load until the HCD film ruptured. The minimum weight load which resulted in HCD film breakage was recorded. Moreover, the elongation at break (%) was calculated by comparing the initial length of the HCD film (before being drawn by the instrument) and

Fig. 1. CA-loaded HCD.

the length at breakage (after bring drawn by the instrument) (Kim et al., 2008a).

2.4.2. Swelling ratio

The square-shaped hydrocolloid samples (2 mm × 2 mm) were subjected to vacuum drying at 60 °C for 12 h. Subsequently, they were soaked in phosphate buffer (pH 7.4) at 37 °C for 24 h. The swelling ratio (SR) was determined using the following formula (Ajji et al., 2005; Kim et al., 2008b): $SR\% = (W_s/W_a) \times 100$, where W_a and W_s are the weights of HCD samples before and after swelling, respectively.

2.5. Drug release test

The release test was carried out using a dialysis instrument (Dialysis tester, Lab fine, South Korea). Fifty milliliters of phosphate buffer (pH 7.4), maintained at 37 °C, was used as the dissolution medium for each sample. The CA-loaded HCD or commercial product was dipped into the dissolution medium and stirred at 50 rpm for 24 h. At pre-determined intervals, 1 ml of the dissolution medium sample was withdrawn, diluted and filtered (0.45 μm). Sampling was compensated for by adding 1 ml of fresh dissolution medium each time. The drug concentration in the filtrate (50 μl) was measured using a Waters 2795HPLC system equipped with a Waters 2795 Separation module and a Waters 2996 photodiode array detector (Waters Co., Milford, MA, USA). The column (4.6 mm I.D × 250 mm, 5 μm) was an Inertsil ODS-3C18 column with an Inertsil ODS-3 guard column (4.0 mm I.D × 10 mm, 5 μm) (GL Sciences Inc., Tokyo, Japan). The mobile phase consisted of acetonitrile/methanol/distilled water (26:24:50, v/v/v) and was filtered (0.45 μm) and eluted at a flow rate of 1.0 ml/min. The concentration of asiatic acid in the eluent was measured at a wavelength of 204 nm (Rafamantanana et al., 2009; Zheng and Wang, 2009). The inter-day and intra-day precision and accuracy were within acceptable limits ($r^2 = 0.999$).

2.6. In vivo wound healing tests

In this preclinical study, four SD rats were used for each wound model. The hair from the back of each anesthetized rat was trimmed at three different places (one spot each for the application of the control, the commercial product and the novel CA-loaded HCD) with an electric trimmer. Then, specific wound models such as excision, infection or abrasion were created at the trimmed areas of each rat. Each rat, inflicted with a particular type of wound, was tested to determine the wound healing effect of the CA-loaded HCDs in comparison with the commercial product.

2.6.1. Excision wound model

At each trimmed area, a full thickness wound was created by excising a portion of the dorsal skin (1.5 × 1.5 mm^2). For sterilization, 70% ethanol was applied. Subsequently, in each rat, the first wound was wrapped with sterile gauze (control), the second wound was covered with the commercial product and the third wound was covered with the CA-loaded HCD. Then, all were fixed with an elastic adhesive bandage (Soft cloth tape®, 3 M, USA) (Hassan et al., 2000; Kim et al., 2008b). Each rat was placed in a separate cage. Digital images of the wound sites were taken at 0, 4, 7, 14, 21 and 29 days.

2.6.2. Infection wound model

A full thickness wound (1.0 × 1.0 mm^2) was inflicted at each trimmed portion on the back skin of each rat. Then, an infection was intentionally initiated in each wound by applying an aliquot of 50 μl of Staphylococcus aureus solution (KCCM 40,050, 3.2×10^8/ml; American Type Collection Culture, Manassas, VA, USA) at the wound site. Like the excision wound model, infectious wounds on the rats were also covered with sterile gauze (control) the commercial product and the CA-loaded HCD. Then, all were secured with an elastic adhesive bandage (Soft cloth tape®, 3 M, USA). Each rat was housed in a separate cage. Images of the wounds were captured at 0, 3, 7 and 10 days using a digital camera.

2.6.3. Abrasion wound model

Three abrasion wounds, each with dimensions of 1.5 × 1.5 mm^2, were induced at the trimmed areas on the back skin of each rat using coarse sandpaper and acetone. For sterilization and cleaning, 70% ethanol was used (Lateef et al., 2005). The wounds were not deep; the wound formation process for this model was ceased upon the observation of fluids and blood oozing out due to superficial skin damage. The wounds on each rat were covered with sterile gauze (control) the commercial product and the CA-loaded HCD. Each rat was accommodated in a separate retaining chamber. Images of the wounds were captured at 0, 1, 2, 3 and 6 days using a digital camera.

2.6.4. Measurement of wound size

Each rat was caged separately. At the predetermined intervals, the size of each wound was measured using a digital camera. The relative wound size reduction was calculated as follows (Kim et al., 2008b): Relative wound size reduction $(\%) = [(A_o - A_t)/A_o] \times 100$, where A_o and A_t are the wound size at the initial time point and time '*t*', respectively. The wound size was assessed using the Adobe® Acrobat® 7 Program.

3. Results and discussion

3.1. Mechanical properties of the CA-loaded HCD

An ideal HCD can withstand the stress resulting from skin movements without rupturing, and provides close and persistent contact between the dressing and skin area around the wound, absorbs exudates from the wound surface, releases a drug consistently and ensures an adequately moist healing milieu at the wound site (Jones et al., 2006). Thus, to develop an ideal HCD, various CA-loaded HCDs were prepared with suitable ingredients such as sodium alginate, polyisobutylene, SIS, PHR and liquid paraffin using hot melting method (Fig. 1). In preliminary study, there were no significant chemical changes in CA contents after the preparation of CA-loaded HCDs (data not shown). In general, the practical wound dressing products are easily and safely manufactured in scale-up via hot melting method, which has no elimination process of organic solvents (Iuchi et al., 2009). The quantity of hydrophilic and hydrophobic polymers in the HCD formulation are of paramount importance in providing suitable mechanical properties such as tensile strength, elongation at break and Young's modulus. Among the ingredients used in this study, sodium alginate was used as the base of the CA-loaded HCD, because it is biocompatible and has excellent hydrocolloid forming properties (Balakrishnan et al., 2005; Thomas, 2000). Alginate hydrogels have shown the ability to retain fluids and maintain a moist environment at the wound site to accelerate the wound healing process (Boateng et al., 2008). Moreover, polyisobutylene and SIS were used as hot melt pressure-sensitive adhesives and elastomers (Hughes and Looney, 1987). These materials impart chemical inertness and excellent resistance to weathering, high temperatures and chemicals. Petroleum resin hydrocarbon (PHR), as a tackifier, can enhance the adhesiveness of the dressing to the skin (Hughes and Looney, 1987). PHR in combination with elastomeric polymers such as polyisobutylene and SIS constitute an ideal adhesive system for HCDs (Zohuriaan-Mehr and Omidian,

Table 1
Composition of CA-loaded HCDs.

Ingredient (wt.%)	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
Drug	1	1	1	1	1	1	1	1	1	1	1
polyisobutylene	23	18	13	10	8	4	–	8	8	8	8
PHR	15	15	15	15	15	15	15	15	15	20	25
SIS	25	25	25	25	25	25	25	27	23	25	25
sodium alginate	24	29	34	37	39	43	47	37	41	34	29
Liquid paraffin	12	12	12	12	12	12	12	12	12	12	12
HCD-forming	No	No	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes

2000). Liquid paraffin also ameliorated the mechanical properties of hydrocolloids in the manufacture of HCDs (Jones et al., 2006).

Firstly, the effect of polyisobutylene on the mechanical properties of CA-loaded HCDs was investigated. As shown in Table 1, formulations I-VII were prepared with various quantities of polyisobutylene and sodium alginate. In the preparation of CA-loaded HCDs, the amounts of drug, PHR, SIS and liquid paraffin were fixed as 1, 15, 25 and 12%, respectively. The tensile strength (Fig. 2A), elongation at break (Fig. 2B) and Young's modulus (Fig. 2C) values of these CA-loaded HCDs were compared. However, formulations I-II prepared a with relatively high concentration of polyisobutylene and VI–VII with a relative very low concentration could not be manufactured due to very high and low viscosity, respectively (Table 1). As the polyisobutylene level was decreased in formulations III–V, the tensile strength and Young's modulus values were decreased, but the elongation at break values were increased; however, these values were not significantly different. Thus, within the concentration ranges used in this study, polyisobutylene scarcely affected the mechanical properties. Even though three formulations showed excellent mechanical properties, formulation V with 8% polyisobutylene was chosen for further study because of its slightly better mechanical properties.

To assess the effect of SIS on the mechanical properties of CA-loaded HCDs, formulations V, VIII and IX were prepared with 1% drug, 8% polyisobutylene, 15% PHR, 12% liquid paraffin, and various amounts of SIS and sodium alginate (Table 1). Formulations V, VIII and IX were not significantly different in terms of tensile strength (Fig. 3A), elongation at break (Fig. 3B) and Young's modulus (Fig. 3C). Similarly, SIS hardly affected the mechanical properties within the concentration ranges used in this study.

Next, to evaluate the effect of PHR on the mechanical properties of CA-loaded HCDs, formulations V, X and XI were prepared with various concentrations of PHR and sodium alginate (Table 1). In the preparation of these CA-loaded HCDs, the concentrations of drug, polyisobutylene, SIS and liquid paraffin were kept constant. Higher concentrations of PHR were associated with greater tensile strength and elongation at break (Fig. 4). Formulation XI showed significantly higher tensile strength (Fig. 4A) and elongation at break values (Fig. 4B) compared to formulations V and X. However, they were not significantly different in terms of Young's modulus (Fig. 4C). Our results suggest that PHR improved both the strength and flexibility of CA-loaded HCDs. Thus, formulation XI composed of CA/polyisobutylene/SIS/PHR/liquid paraffin/sodium alginate at the weight ratio of 1/8/25/25/12/29 was considered to be the optimum formulation of CA-loaded HCD due to its excellent mechanical properties.

Like other pharmaceutical preparations, the release of a drug from the CA-loaded HCD is very important for producing its intended effects (Bolhuis et al., 1997). In this study, a dialysis method for release was used, because the CA-loaded HCD was not a transdermal but a local pharmaceutical product for treatment of external wound (Kim et al., 2008a,b). The preliminary release test was carried out with the selected CA-loaded HCD, resulting in no release of the drug. Thus, a disintegrant was added to increase drug release from the CA-loaded HCD. In this study, the swellable disintegrants croscarmellose sodium, sodium starch glycolate and crospovidone were assessed (Balasubramaniam et al., 2008; Bussemer et al., 2003; Caramella et al., 1990; Setty et al., 2008).

Three CA-loaded HCDs were prepared with 2% disintegrants and 27% sodium alginate, and their swelling ratios were investigated. Croscarmellose sodium significantly improved the swelling of CA-loaded HCD compared to the other disintegrants (Fig. 5A). Furthermore, the swelling ratios were investigated with the CA-loaded HCDs prepared with various concentrations of croscarmellose sodium (Fig. 5B). Up to 2%, croscarmellose sodium largely increased the swelling ratio, with no significant change above this level. Moreover, the CA-loaded HCD without croscarmellose sodium poorly released the drug within this period of time, but the HCD with 2% croscarmellose sodium showed about 27% drug release in 24 h (Fig. 5C). Thus, croscarmellose sodium greatly improved drug release. Therefore, the formulation composed of CA/polyisobutylene/SIS/PHR/liquid paraffin/sodium alginate/croscarmellose sodium at the weight ratio of 1/8/25/25/12/27/2 was selected as the optimal CA-loaded HCD for further study.

Prior to in vivo wound healing experiments, the mechanical properties of the CA-loaded HCD were compared to the commercial product (Duoderm™, Convatec Co.). The CA-loaded HCD had a significantly higher tensile strength (0.034 ± 0.002 *vs.* 0.028 ± 0.004, N/mm^2), elongation at break (216.0 ± 6.1 *vs.* $183.0 \pm 6.9\%$) and swelling ratio (249.9 ± 8.9 *vs.* $158.7 \pm 9.5\%$),

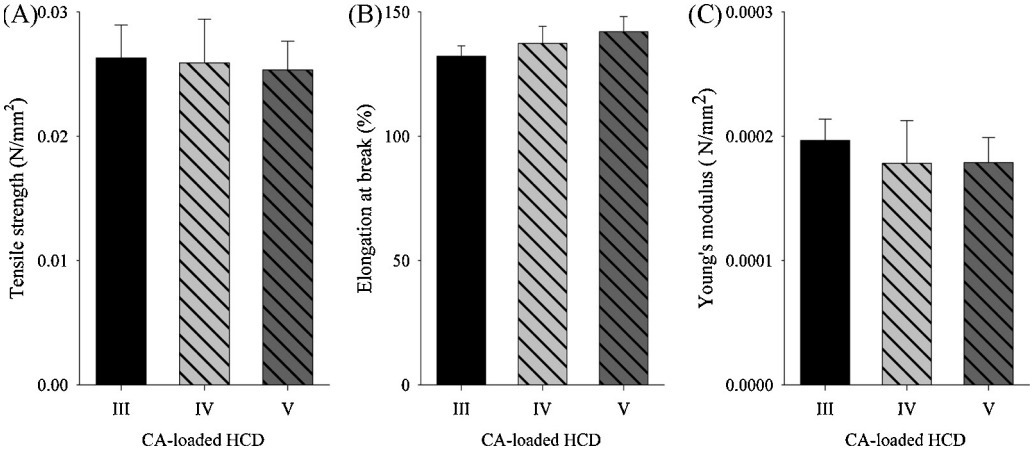

Fig. 2. Effect of polyisobutylene on the mechanical properties of CA-loaded HCDs: (A), tensile strength; (B), elongation at break; (C), Young's modulus. Each value represents the mean ± S.D. ($n = 3$).

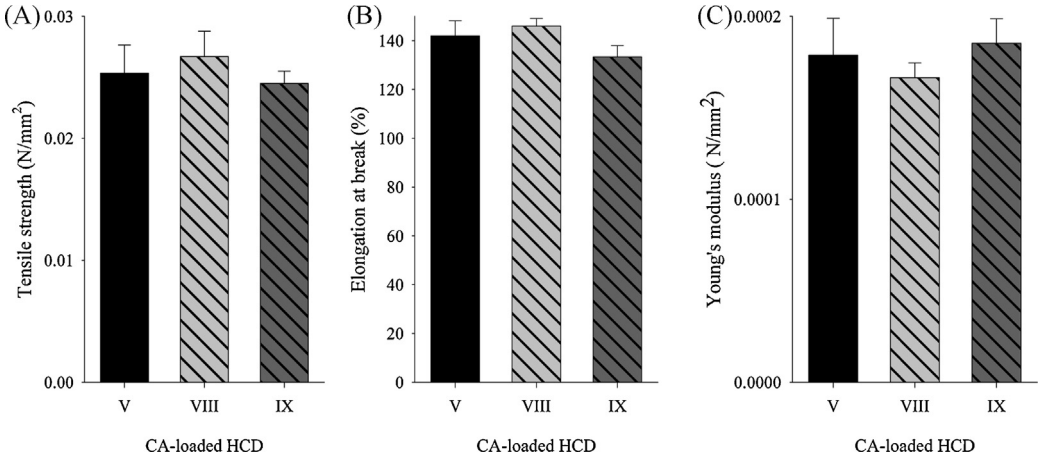

Fig. 3. Effect of SIS on the mechanical properties of CA-loaded HCDs: (A), tensile strength; (B), elongation at break; (C), Young's modulus. Each value represents the mean ± S. D. ($n = 3$).

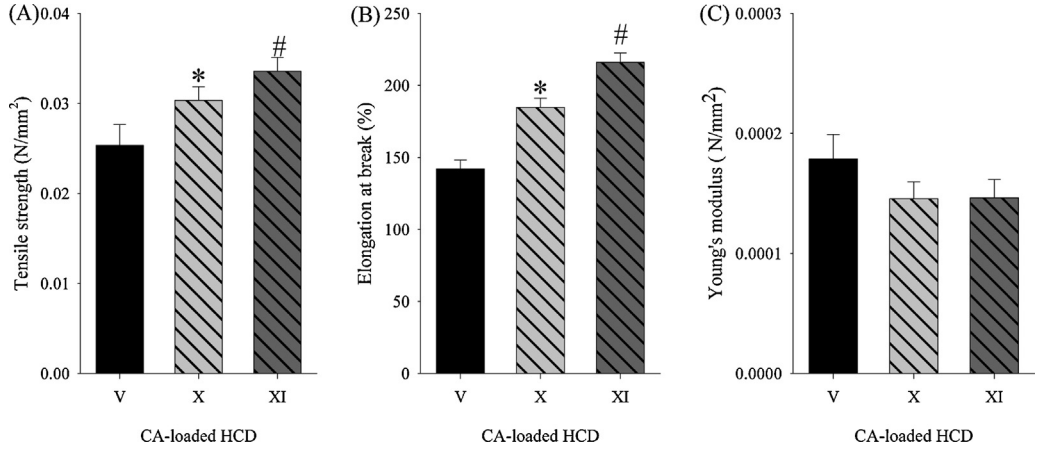

Fig. 4. Effect of PHR on the mechanical properties of CA-loaded HCDs: (A), tensile strength; (B), elongation at break; (C), Young's modulus. Each value represents the mean ± S. D. ($n = 3$). $^{*}P < 0.05$ compared with formulation V. $^{\#}P > 0.05$ compared with formulation V and X.

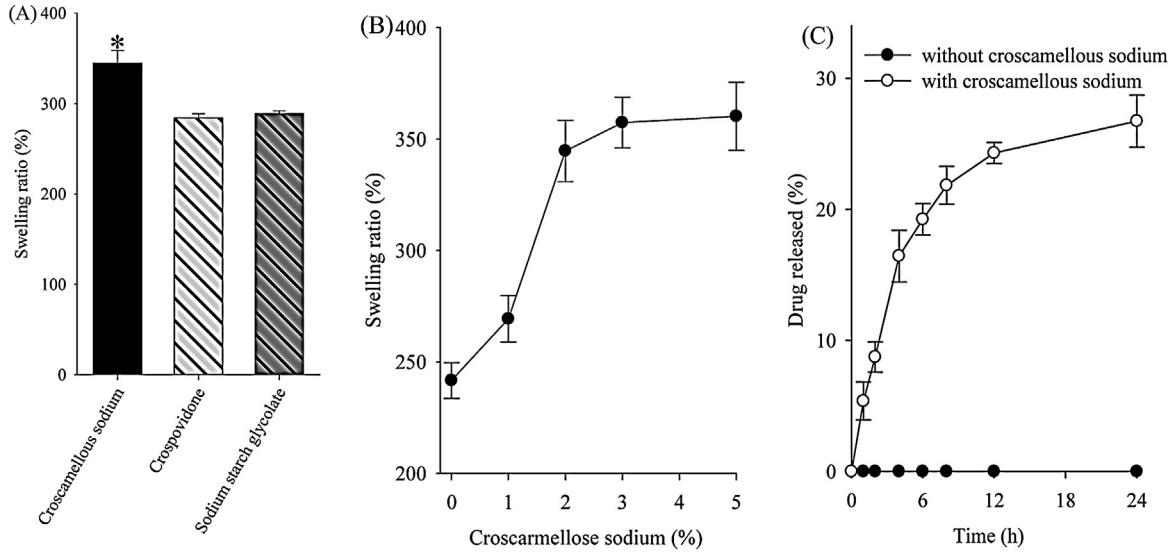

Fig. 5. Effect of disintegrants: (A), Comparative swelling ratios of CA-loaded HCDs prepared with different disintegrants; (B), effect of croscarmellose sodium on the swelling ratios of CA-loaded HCDs; (C) release of the drug from CA-loaded HCDs with or without croscarmellose sodium. Each value represents the mean ± S.D. ($n = 3$ or 6). $^{*}P < 0.05$ compared with other disintegrants.

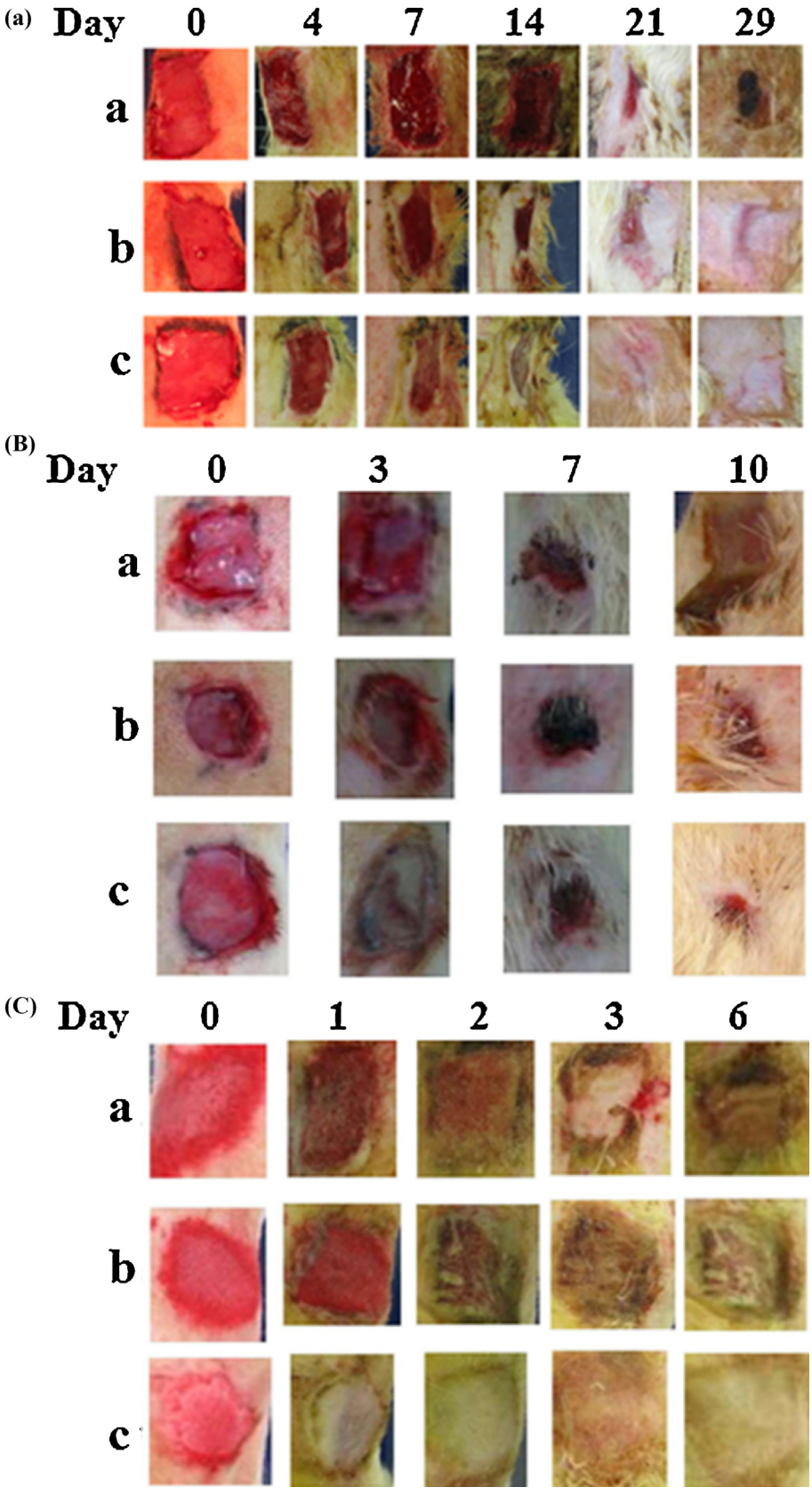

Fig. 6. Representative images of the excision wound model (A), infection wound model (B) and abrasion wound model (C): (a), control; (b), commercial product; (c), CA-loaded HCD.

and a lower Young's modulus (1.46 ± 0.10 *vs.* 1.55 ± 0.25, $X10^{-4}$ N/mm^2) compared to the commercial product ($P > 0.05$). Thus, the CA-loaded HCD had better swelling capacity and flexibility than the commercial product.

3.2. In vivo *wound healing*

The *in vivo* wound healing potential of the CA-loaded HCD was assessed in comparison with the commercial product using the abrasion, excision and infection wound models. As the control, a wound covered with gauze and without other treatment was used. The wound healing process is relatively slower in diabetics than in normal individuals; therefore, the assessment of the wound healing potential of a dressing is most suitable in a diabetic wound model (Lateef et al., 2005; Warner et al., 2008). Thus, diabetes was induced in rats prior to the induction of the wound models. The rats with a blood glucose level >250 mg/dl were used in these experiments. For each wound, the healing rate (%) was determined by the relative wound size reduction (%).

Firstly, the wound healing process in the excision wound models was examined over a period of 1 month. Representative images of the reduction in the excision wound size with the passage of time (at 4, 714, 21 and 29 days) are displayed in Fig. 6A. As compared to the control (untreated wounds) (Fig. 6A–a), the wound sizes were reduced by the commercial product (Fig. 6A–b) and the CA-loaded HCD (Fig. 6A–c) owing to accelerated epithelialization.

In the infection wound model, the wounds were inoculated with S. aureus after wound formation (Boonkaew et al., 2014; De Cicco et al., 2014). Subsequently, the CA-loaded HCD and commercial product were applied to these infected wounds. Representative images of the wounds on day 0, 3, 7 and 10 are given in Fig. 6B. Wound healing progression in the infection wound model was observed over a period of 10 days. On day 3, inflammation was noted in all wounds; however, severe manifestations such as inflammation with hemorrhage were seen only in the control. As compared to the control (Fig. 6B–a), wound sizes were decreased by the commercial product (Fig. 6B–b) and the CA-loaded HCD (Fig. 6B–c) due to accelerated epithelialization. The wound healing process in the abrasion wound model was studied over a period of 6 days. Representative images of the abrasion wounds on day 0, 1, 2, 3 and 6 are presented in Fig. 6C. As compared to the control (Fig. 6C–a), the commercial product (Fig. 6C–b) and CA-loaded HCD (Fig. 6C–c) showed accelerated healing.

The wound healing profiles are shown in Fig. 7. In the excision wound model, wound healing with the commercial product and the CA-loaded HCD was significantly faster than in the control ($P < 0.05$). In particular, on each day, the CA-loaded HCD showed better wound healing than did the commercial product; however, these interventions were not significantly different ($P > 0.05$). On day 29, wound healing in rats treated with the control, commercial product and CA-loaded HCD was about 71, 85 and 95%, respectively (Fig. 7A). In the infection wound model, wound healing with the commercial product and the CA-loaded HCD was significantly enhanced compared to the control ($P < 0.05$) (Fig. 7B). In particular, on each day, wound healing with the CA-loaded HCD was better than with the commercial product; however, these interventions were not significantly different ($P > 0.05$). At day 10, wound healing in the control, commercial product and CA-loaded HCD treated rats was about 37, 58 and 75%, respectively (Fig. 7B). Furthermore, in the abrasion wound model, wound healing (%) with the commercial product and the CA-loaded HCD was significantly better than in the control ($P < 0.05$) (Fig. 7C). In particular, initial wound healing (on day 1 and 2) with the CA-loaded HCD was significantly better than with the commercial product ($P < 0.05$). On day 6, wound healing in rats treated with the control, the commercial product and CA-loaded HCD was about 60, 72 and 90%, respectively (Fig. 7C).

Based on these findings, the wound size gradually reduced over time due to wound closing. Wound healing was faster and better in the decreasing order: CA-loaded HCD $\geq$ commercial product > control. As compared with the control, quicker and better wound healing in the CA-loaded HCD and commercial product likely occurred due to the moist environment at the wound site. A moist environment around a wound has a profound beneficial impact on the physiological process of wound healing (Kim et al., 2008b; Remunan-Lopez and Bodmeier, 1997) as it makes the wound soft and facilitates the migration of fibroblasts and keratinocytes and the distribution of cell growth factors and cytokines (Kim et al., 2006, 2008a). Thus, moist wound healing accelerates epithelialization and the various stages of wound healing. In our study, although there was no significant difference ($P > 0.05$) in the healing profiles of the CA-loaded HCD and the commercial product, the former exhibited better healing potential. This positive healing effect of the CA-loaded HCD might be attributed to the presence of sodium alginate in its composition. Sodium alginate has been reported to accelerate the wound healing process in some investigations (Chiono et al., 2008; Cho and Lo, 1998). Thus, the

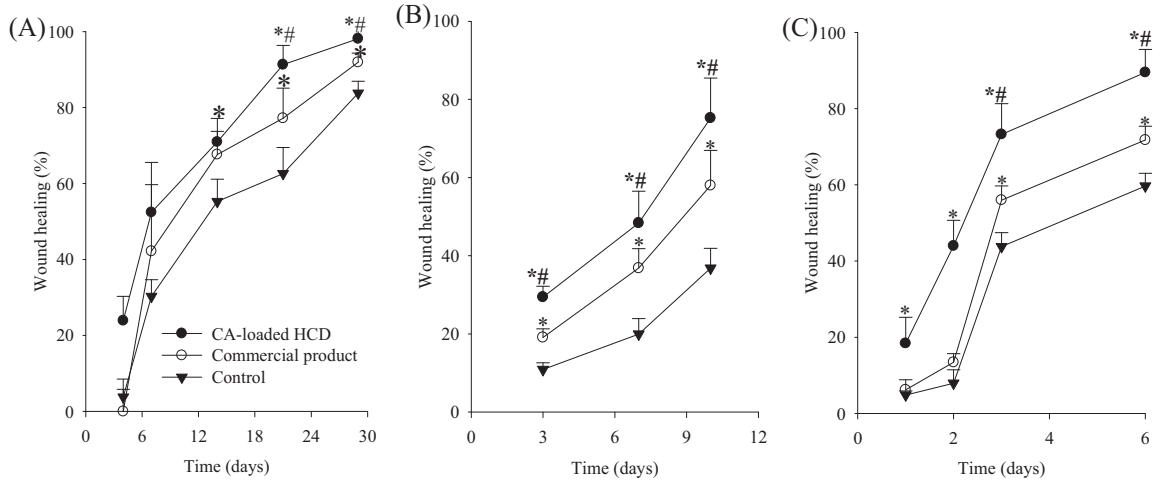

Fig. 7. Wound healing effect of the CA-loaded HCD: (A), excision wound model; (B), infection wound model; (C), abrasion wound model. Each value represents the mean ± S. D. ($n = 4$). * $P < 0.05$ compared with the control. $^{\#}P > 0.05$ compared with the commercial product.

CA-loaded HCD enhanced wound healing as compared with the commercial product.

4. Conclusion

A novel CA-loaded HCD consisting of CA/polyisobutylene/SIS/PHR/liquid paraffin/sodium alginate/croscarmellose sodium at a weight ratio of 1/8/25/25/12/27/2 showed excellent swelling, drug release and mechanical properties. As compared to the commercial product, it enhanced the healing effect in excision, infection and abrasion wounds in rats. Thus, this CA-loaded HCD could be a potential candidate for the treatment of various wounds. To develop a practical CA-loaded HCD, further study on its clinical test will be performed with human subjects.

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