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Hydrogel sheets of chitosan, honey and gelatin as burn wound dressings

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ABSTRACT

A hydrogel sheet composed of chitosan, honey and gelatin (HS; 0.5:20:20, w/w) was developed as a burn wound dressing. HS showed powerful antibacterial efficacy up to 100% to *Staphylococcus aureus* and *Escherichia coli*, significantly superior to chitosan and honey used separately. A series of toxicological evaluations demonstrated that HS is not toxic and not irritant to skin and body. An animal burn model was performed on the back of New Zealand rabbit, and treated, respectively, with HS, MEBO® ointment (Shantou MEBO Pharmaceuticals Co., Ltd., Guangdong, China) and sterile gauze. The macroscopic image and histopathology were examined. The results showed that HS had a significant effect on wound contraction with the shortest treatment duration of 12 days compared to MEBO® ointment and no treatment. Histological examination revealed that HS-treated burn wound was repaired with intact epidermis on day 12, but the wound treated with MEBO did not completely heal. Therefore, HS demonstrated its potential as a treatment.

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1. Introduction

An ideal dressing should create a moist wound healing environment, absorb excess exudate, allow gaseous exchange and be removed easily without trauma to the wound. It also should be antimicrobial, nontoxic and biocompatible. These performances are primarily attributed to the functional characteristic of the material and the microenvironment created (Stashak, Farstvedt, & Othic, 2004). It appears that no single material can achieve all the requirements for all the stages of the wound healing process. So several commonly used biomaterials, such as chitin/chitosan, alginate and gelatin are presently adopted to fabricate wound dressings (Ibrahim, 2009; Knill et al., 2004; Lee, Churey, & Worobo, 2008). Similarly, honey has been applied to wound treatment since ancient times (Moghazy et al., 2010; Subrahmanyam, 1998; Visavadia, Honeysett, & Danford, 2008; Yusof, Hafiza, Zohdi, & Bakar, 2007). Therefore, these materials are all suitable candidates for wound dressings.

Chitosan has been proved to have desirable qualities, such as hemostasis, bacteriostasis, biocompatibility, and biodegradability properties (Fan, Hu, & Shen, 2009; Muzzarelli, 1993; Pillai, Paul, & Sharma, 2009; Ueno, Mori, & Fujinaga, 2001). Now chitosan has been used in a wide range of biomedical applications (Kim et al., 2008; Lu, Gao, & Gu, 2008; Muzzarelli, 2009). Various studies have suggested that chitosan seems to be an excellent dressing material for wound healing. It has been widely used for wound dressings in the form of hydrogel (Boucard et al., 2007; Murakami et al., 2010; Ribeiro et al., 2009), fiber (Kossovich, Salkovskiy, & Kirillova, 2010; Watthanaphanit, Supaphol, Tamura, Tokura, & Rujiravanit, 2008; Zhou et al., 2008), membrane (Azad, Sermsintham, Chandrkrachang, & Stevens, 2004; Mizuno et al., 2003; Pei, Chen, Li, & Zhou, 2008; Thomas, Yallapu, Mohan, & Bajpai, 2009; Zhang, Yang, & Nie, 2008), scaffold (Sudheesh Kumar et al., 2010; Zhang et al., 2009) and sponge (Denkbas, Ozturk, Ozdem, Kecec, & Agalar, 2004; Lee et al., 2000). To improve the wound healing properties, chitosan-based materials have been developed with different types of polymers such as alginate, gelatin, poly(vinyl alcohol), polyethylene glycol diacrylate, γ-poly(glutamic acid) and 2-hydroxyethyl methacrylate (Jayakumar, Prabaharan, Sudheesh Kumar, Nair, & Tamura, 2011). The composite nature endows chitosan-based materials with the desired properties for wound healing applications. However, a hydrogel might be the most useful form of chitosan for wound dressings, which currently was believed to possess most of the properties of an ideal wound dressing (Stashak et al., 2004). It would be more effective in hydrogel form for chitosan to protect and contract the wound in a suitably moist healing environment (Obara et al., 2003). Additionally the moist healing environment and accelerating effects of chitosan-based materials in other forms cannot be exploited due to the relatively low interaction between the wound and these healing agents (Murakami et al., 2010).

Moreover, honey should also be taken into account. It is a natural supersaturated sugar solution with approximately 17% water, including fructose, glucose, maltose, sucrose and other types of

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Table 1
Preparation data of chitosan/honey/gelatin hydrogel. Unit: g.

Sample	HS	HS-1	HS-2	HS-3	HS-4
Chitosan	0.5	0.25	0. 5	0	0.5
Honey	20	20	10	20	0
Gelatin	20	20	20	20	20

carbohydrates (Lee et al., 2008; Tshukudu, Walt, & Wessels, 2010). The composition of honey depends largely on its floral source which plays an important role on its biological properties. While not all honeys are equally effective for wound healing (Basualdo, Sgroy, Finola, & Marioli, 2007). Even when it was ineffective, honey never resulted in the emergence of resistant strains (Moghazy et al., 2010). In fact, honey has been commercially used for wound dressings due to its suitability for all stages of wound healing. A honey hydrogel dressing has also been developed for enhanced wound healing (Yusof et al., 2007). With regard to the mechanism of antibacterial activity, there are great differences between honey and chitosan, which may be transferred into more significant advantages on preventing infection by their cooperation. Recently, Jia, Lu, and Xuan (2010) reported VAP (velvet antler polypeptide)–chitosan–honey suspension applied on decubitus ulcer. The honey based suspension was found to promote wound healing with the scar falling off early and absolutely. It suggested that the blend of chitosan and honey could be more promising for wound healing application.

Additionally, gelatin, a biocompatible protein obtained by partial degradation of collagen, has always been used together with chitosan to prepare composite film for biomedical applications for its low cost and excellent functional and filmogenic properties (Nagahama et al., 2009; Pereda, Ponce, Marcovich, Ruseckaite, & Martucci, 2011; Rivero, García, & Pinotti, 2009). Besides, gelatin is of advantage to absorb excess exudates because of its excellent ability to absorb water more than 5–10 times as weight as itself. In this study, therefore, chitosan, honey and gelatin were selected to prepare hydrogel sheets (coded as HS) as wound dressings. The properties of the hydrogel sheet, such as antibacterial activity, nontoxicity and wound healing, were investigated.

2. Materials and methods

2.1. Materials

Chitosan was purchased from Kelong Chemical Industry, Ltd. (Chengdu, China), deacetylation degree was ~85–93% and molecular weight was ~100–300 kDa. Sunflower honey untreated was obtained from local market (Chongqing, China). Bovine gelatin type B provided by Tianjin Ruijinte Chemical Co., Ltd. (Tianjin, China). All other chemicals used were of analytical grade for these studies.

2.2. Preparation of hydrogel sheets

A mass of 0.5 g chitosan powder was added into 19 ml distilled water, and then 0.5 g glacial acetic acid was dropped into the water with stirring until chitosan dissolved completely. A mass of 20 g honey was diluted 50:50 (w/w) and 20 g gelatin was dissolved in the same weight of distilled water at 60 °C. After that, aqueous gelatin was agitated at 40 °C, meanwhile the chitosan solution and diluted honey were mixed up with the aqueous gelatin in the different ratios (Table 1) and distilled water was added up to 100 g. Then the mixed fluid was spread on a watch-glass at a thickness of 2–4 mm, followed by standing and cooling to room temperature to form hydrogel. Next, the obtained hydrogel sheet was sterilized with ultraviolet rays (aseptic operating platform Type SW-CJ-1BU; Suzhou Antai Air Tech Co., Ltd., Jiangsu, China) for 45 min.

2.3. Swelling behaviors

The swelling studies of the samples were carried out according to the method described by Yang et al. (2010) with some modifications. The samples of a cylinder shape (diameter 30 mm, thickness 4 mm) were dried in the vacuum oven at 37 °C for 24 h and weighted (W_0). Then the samples were immersed in distilled water at 25 °C for 24 h and weighted (W_{24}). The equilibrium swelling in water (ESW) was calculated using the following equation:

$$\%\,\mathrm{ESW} = \frac{W_{24} - W_0}{W_0} \times 100$$

The swelling properties of the hydrogels in phosphate buffer solution (PBS) (0.15 mol L^{-1}, pH = 7.2) were measured by determining the weights of the swollen at 37 °C at various time points. The swelling degree in PBS (SDP) was calculated by the following equation:

$$\%\,\mathrm{SDP} = \frac{W_t - W_i \times a}{W_i \times a} \times 100$$

where W_t is the weight of the hydrogel swollen in PBS at time t, W_i is the initial weight of hydrogel samples, and 'a' is equivalent to the concentration of components of the samples, which approximately equal 0.4 for HS, HS-1 and HS-3, 0.3 for HS-2, and 0.2 for HS-4 in this study.

2.4. Mechanical properties

The mechanical properties of the hydrogel samples were determined by conducting compressive strength tests using a texturometer TA.XT2i—Stable Micro systems (Surrey, England) equipped with a SMS P/0.5R plunger of 12.5 mm diameter. The samples were compressed 4 mm with a constant speed of 0.5 mm/s, and the compression force was expressed in grams.

2.5. Antibacterial activity test

Escherichia coli and *Staphylococcus aureus* were used as the test organisms, which were prepared from fresh colonies on tryptic soy agar (TSA). One loopful of the bacteria was inoculated in a test tube, which shook in an air-bath shaker at 37 °C for 18 h. The cultures of *E. coli* and *S. aureus* containing approximately 10^8 CFU/mL were prepared and used for the antibacterial activity test.

The inhibition rates of the test materials were determined using agar plates. For this purpose, the samples were prepared and kept the form of fluid at 38 °C. The peptone culture plates were prepared, in which 200 μL solution of bacterial suspension was spread uniformly and then 150 μL fluid of sample. All the plates were incubated at 37 °C for 24 h. Then the plates were taken out of the incubator, and the inhibition rate was calculated.

The inhibition rate was defined as (Zheng & Zhu, 2003):

$$\%\,\text{inhibition rate} = \frac{N_0 - N_1}{N_0} \times 100$$

N_0 and N_1 are the number of colonies on the plates before and after inhibition, respectively.

2.6. Morphology

Morphologies of the surface and the cross-section of the lyophilized HS samples were studied by scanning electron microscope (SEM, Hitachi S-4800, Japan) with an accelerating voltage of 5 kV after gold coating. SEM observations were performed using the followings magnifications: 1000×, 2000× and 5000×.

2.7. Animals

Kunming mice (20 ± 2 g) and New Zealand rabbits (2.5 ± 0.5 kg) were obtained from the Center for Laboratory Animals, Chongqing Medical University, China. All animals were maintained in accordance with the university guidelines for animal experimentation.

2.8. Toxicological evaluation

The animal toxicology evaluation of HS was assessed according to the ISO standard. Acute oral toxicity test and dermal irritation test were arranged according to ISO 10993-11: 2006 and ISO 10993-10: 2002, separately.

2.8.1. Acute oral toxicity test

The maximum tolerance dose test was carried out on mice at a dose of 10 g/kg b.w. The test material was prepared by diluting 5 g HS with 15 mL double distilled water at 50 °C. Twenty healthy Kunming mice (half male and half female) were separated into two groups. Each mouse received intragastrical injection of the test material in two doses (20 mL/kg b.w.) at an interval of 4 h. Then they had free access to food and clean drinking water during the 2 weeks of acclimatization and throughout the experimental period. The toxic symptoms and the death of mice were recorded. In addition, another ten untreated mice (half male and half female) were used as control group.

2.8.2. Dermal irritation test

Four New Zealand rabbits (male) were shaved with an electric razor over an area of 3 cm × 3 cm on both sides of the spine, one for experiment, another for control, respectively. After 24 h, the experimental area was applied with 0.5 g HS and coated with two layers of gauze (2.5 cm × 2.5 cm) and a layer of cellophane for 4 h, respectively. Then the gauze was removed and warm water was used to rinse the residue of samples. The reaction of skin such as erythema and dropsy and their extent were recorded at 1 h, 24 h and 48 h, respectively.

2.9. In vivo wound healing experiments

2.9.1. Establishment of skin burns

Each rabbit that shaved over an area of 3 cm × 3 cm on the dorsal side, was anesthetized by an ear marginal intravenous injection of 30 g/L sodium pentobarbital, at a dose of 30 mg/kg body weight, respectively. Next, the surgical area was burned with boiled gauze for 15 s, which had 35 layers and over an area 3 cm × 3 cm. Then the second degree burn wound was established (Liu, Yu, Zhang, Zhou, & Tie, 2009).

2.9.2. Treatment of burns

The burned rabbits were divided into three groups. The treatment applied to each of the tested samples on the wound was carried out shortly after burn wound was produced and rinsed with 75% alcohol. Group I animals were treated with HS; Group II with MEBO® moisture ointment; Group III received no treatment. Once daily for 12 days, the test samples were applied after cleaning the wound with a dilute solution of Dettol®. The burns were then covered with Boat Brand® absorbent gauze (Chongqing 9th Pharmaceutical Factory, Chongqing, China), bandaged with OYEAH® Sheer Bandage (Hangzhou Outuopu Biological Technology Co., Ltd., Zhejiang Province, China), and allowed to heal. The bandages were replaced every other day. Similar procedures were also applied to the control group.

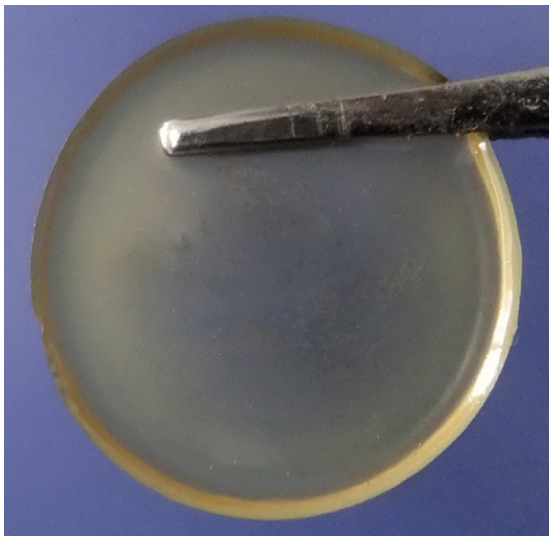

Fig. 1. Photographic appearance of the hydrogel sheet HS.

2.9.3. Visual observation of burn wound

The burn wound size measurements taken at the time of surgery and at the time of biopsy were used to calculate the percent wound contraction, using Eq. (1) (Ibrahim, 2009):

$$\%\,\text{wound contraction} = \frac{A_0 - A_t}{A_0} \times 100$$

where A_0 is the original burn wound area, and A_t is the burn wound area at the time of biopsy.

The time of complete healing of burns was tracked also. It was identified by the time that no scab and no ulcer were present on the burn site.

2.9.4. Histological examination

The excised burn sites were fixed with formalin for 6 h, followed by rinsing and dehydration, and then embedded in paraffin. Thick sections (4–7 μm) were stained with haematoxylin–eosin stain and photographed under 100× magnification.

2.10. Statistical analysis

All data are presented as the mean ± the standard deviation (s.d.). Differences between means were analyzed for statistical significance using Student's *t*-test. *p*-Values < 0.05 were considered significant.

3. Results

3.1. Preparation of hydrogel sheets

The hydrogel sheets were prepared using chitosan and honey with gelatin at various contents (Table 1), which were uniform, soft and semi-transparent. The sheets exhibited golden yellow color and honey smell except HS-4 (without honey). All the samples were easily removed from the glass plate and showed smooth surface (HS as an example shown in Fig. 1).

3.2. Swelling studies

The swelling behaviors of the samples were investigated. Fig. 2a shows the equilibrium swelling in water (ESW) of the samples, which indicated the water retention ability of the samples. ESW of the hydrogels prepared with the same content of 20 wt% honey, i.e., HS, HS-1, and HS-3 showed the similar values at bottom. With

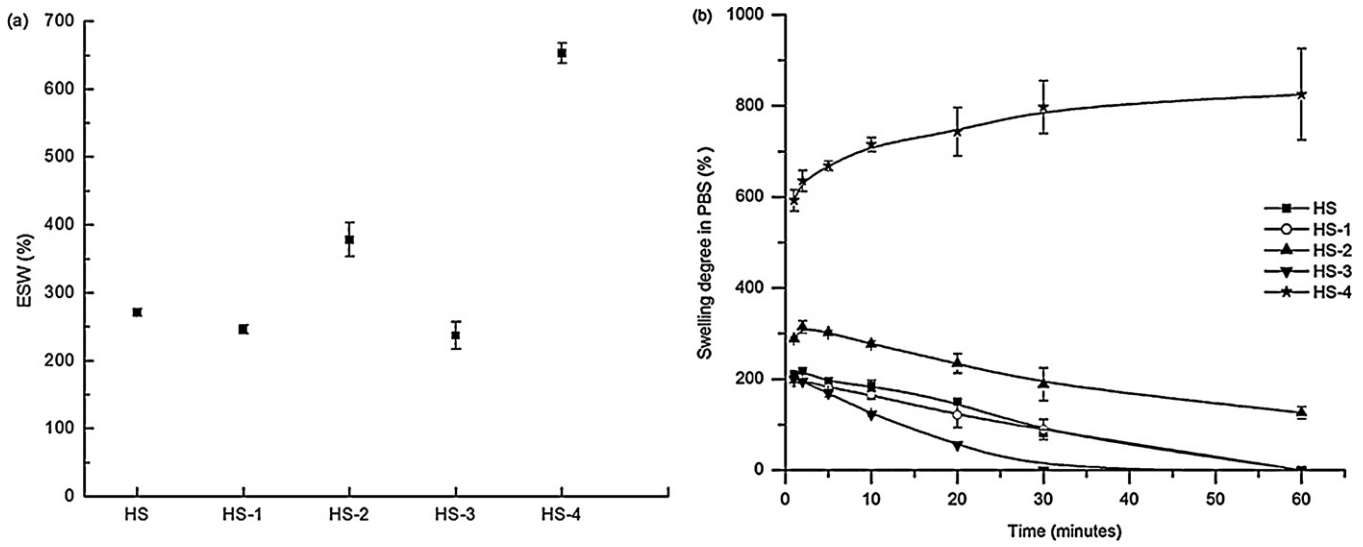

Fig. 2. Swelling behaviors of the samples: (a) equilibrium swelling in water (ESW) and (b) swelling degree in PBS (SDP) ($n = 3$).

the decrease of honey, ESW of the hydrogels increased significantly and the maximum value was reached by HS-4 prepared without honey. Fig. 2b shows the swelling degree in PBS (SDP) of the samples against time evaluating the capacity to absorb exudate. SDP of the samples correlated to the content of honey as the same as ESW. The samples swelled rapidly and reached equilibrium within 5 min except HS-4, and SDP of which gradually decreased against time. On the contrary, HS-4 yielded the highest SDP and get equilibrium at 30 min.

3.3. Mechanical properties

The Young's modulus of the hydrogel samples is shown in Fig. 3. As it shown, the hydrogel blends of chitosan and gelatin (HS-4) yielded the highest modulus, and the lowest modulus also obtained while honey was mixed up with the content of 10 wt% (HS-2). In fact, the modulus of the samples with or without chitosan decreased substantially due to the presence of honey as compared with HS-4.

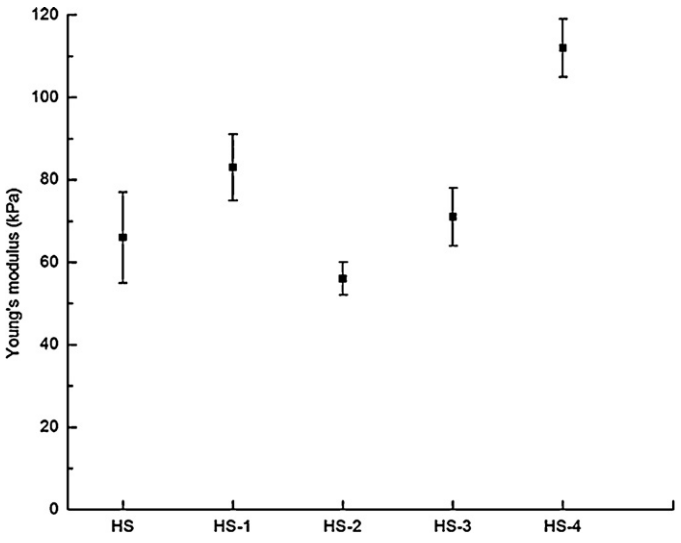

Fig. 3. Young's moudlus of the hydrogel samples ($n = 3$).

3.4. Antibacterial activity

The inhibition rates to *S. aureus* and *E. coli* of the test materials after 24 h were shown in Fig. 4. Neither the inhibition rate of HS-3 (without chitosan) nor the HS-4 (without honey) surpassed 50%. However, the inhibition rates to both *S. aureus* and *E. coli* were enhanced greatly to 100% when HS-3 or HS-4 was given the lack part to be HS was used (Fig. 4). When the concentration of chitosan or honey reduced the antibacterial activities of the samples, e.g., HS-1 and HS-2 decreased in certain extent. The inhibition rates to *S. aureus* and *E. coli* of HS at 48 h and 72 h continuously kept up to 100% (data not shown). This indicated that neither chitosan nor honey could independently have as good an effect on antibacterial activity as they are mixed together.

3.5. Morphology

The SEM images of the hydrogel sheet HS with different water contents were shown in Fig. 5. The lyophilized HS with 40% water exhibited a smooth surface (Fig. 5a) and a similarly smooth cross-section (Fig. 5b). The large pores were not observed on both the surface and the cross-section of the HS. The morphology of the swollen HS with 130% water changed obviously. The surface of the swollen HS wrinkled (Fig. 5c) and the cross-section became

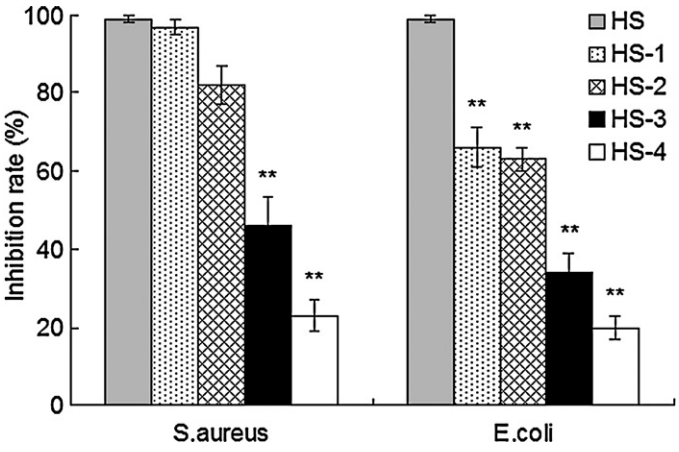

Fig. 4. Comparison of the inhibition rate of hydrogel samples ($n = 3$). $^{**}p < 0.005$.

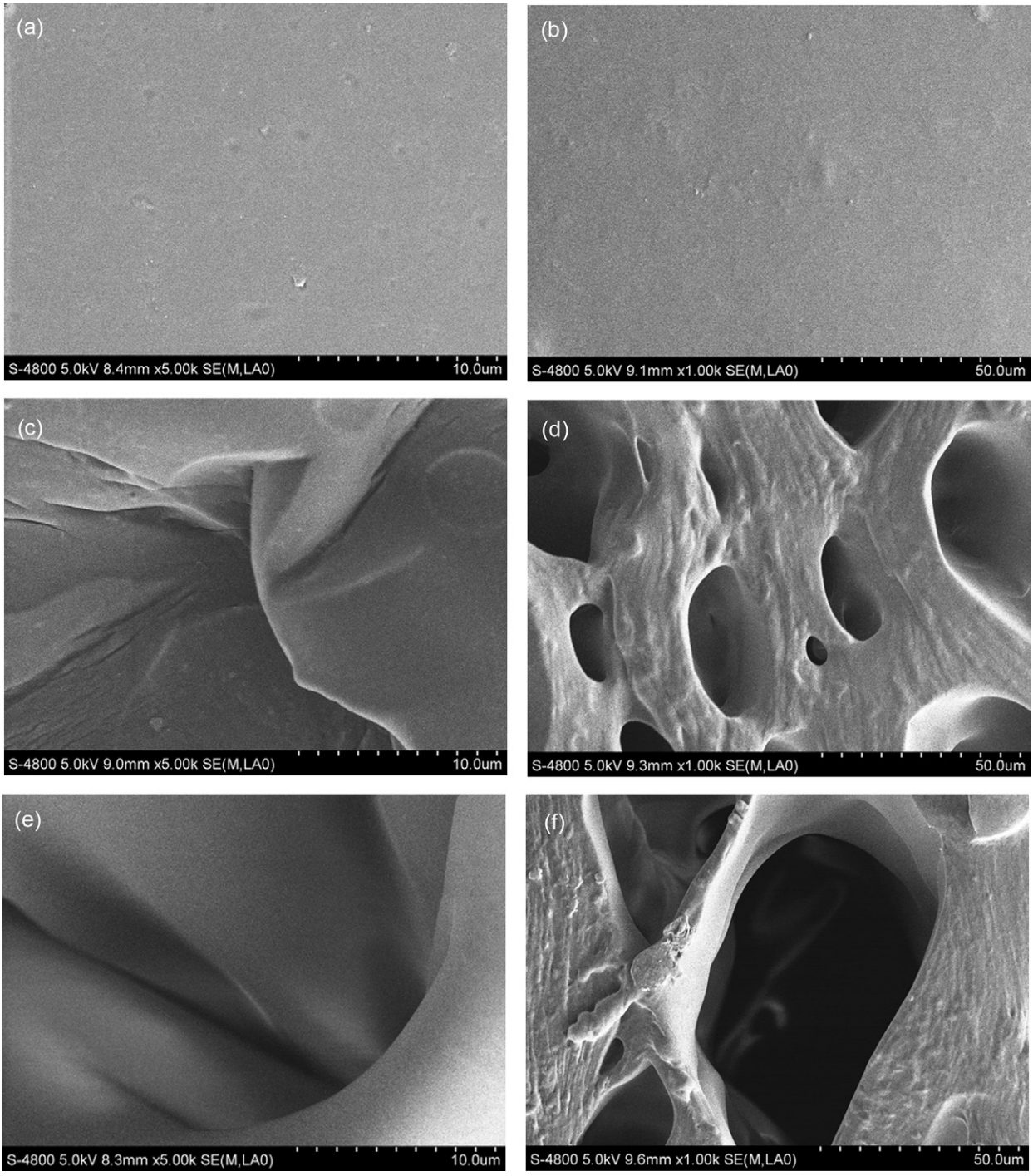

Fig. 5. SEM images of the lyophilized hydrogel sheet HS with different water content. Surface morphology of HS with 40% water (a), 130% water (c), 200% water (e), and (b), (d) and (f) the corresponding cross-section morphology.

microporous (Fig. 5d). The HS swelled with 200% water showed a collapsed surface (Fig. 5e) and porous cross-section with reduction of the pore number and great increase of the pore size (Fig. 5f).

3.6. Toxicological evaluation

Every mouse was observed for 2 weeks after injection. No mouse died and no toxic symptoms occurred. There was no significant difference between the experimental group and the control group. Therefore, the result illustrated that HS did no harm to the animals tested.

The reaction of skin irritation was observed at 1, 24 and 48 h. No erythema and dropsy was observed on the skin at the various time points. The results showed that HS causes no irritation to skin. So it indicated that HS is a safe material suitable for burn wound dressing.

3.7. Visual observation of burns

To ascertain HS's wound healing acceleration superiority, HS was compared to a commercially available MEBO® ointment, which is used to treat a number of bacterial skin infections and burns. The

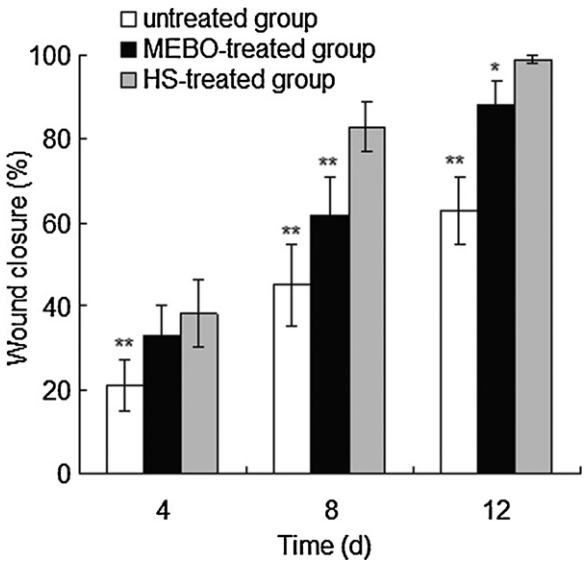

Fig. 6. Wound closure at 4, 8 and 12 days post-wounding ($n = 6$). $^{**}p < 0.005$; $^{*}p < 0.01$.

rate of wound contraction was evaluated by determination of the unclosed area as a function of time (Fig. 6). Compared with the control and MEBO-treated burns, HS-treated burns contracted at the highest rate. On day 4, there was no significant difference between MEBO and HS-treated burns, which were significantly higher than the control. On day 8, wound contraction of the HS-treated group increased to 80%, 20% higher than the MEBO-treated group. On day 12, burns treated with HS were complete healed with intact epidermis. In contrast, there was still about 15% wound area unclosed on MEBO-treated burns. Control burns healed even more slowly and about 63% wound closure was achieved.

A comparison of burn wound healing days recorded in Fig. 7 revealed that healing time with HS was about 12 days. However, the MEBO-treated group and the control group required 14 days and 17 days to completely recover the burns, respectively. It confirmed that HS greatly accelerated the rate of wound contraction.

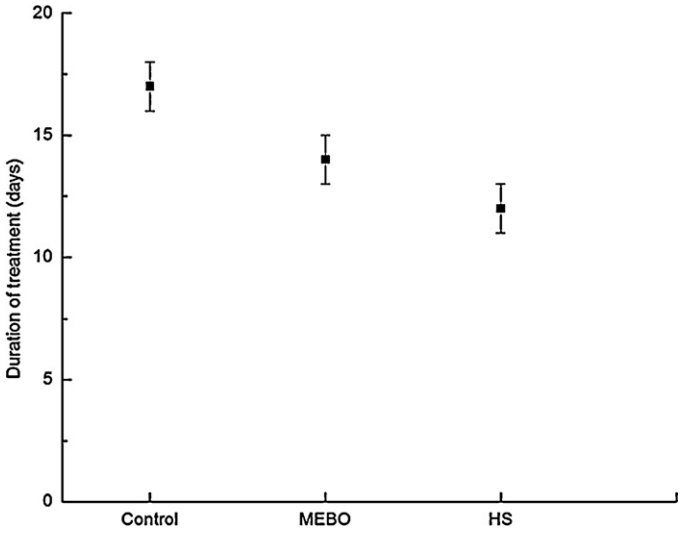

Fig. 7. Time of complete healing after wounding ($n = 6$).

3.8. Histological examination on healing burns

The healing pattern of burns was studied by examining the histology of burn sites 12 days post-burning (Fig. 8). In the control group, an ulcer (u) still existed at the center of the burn wound about an area one-third of the burn site, on which covered a large scab (s) (Figs. 6 and 8A). The untreated burn wound was infected and many inflammatory cells (inf) could be observed at the burn site. In addition, hair follicles basically disappeared (Fig. 8A). Compared with the control group, the medical group (MEBO) and the experimental group (HS) obviously promoted epidermis (e) growth and the wound contraction (Fig. 8B and C). As for MEBO-treated burns, the scab had just fallen off naturally, but there were still small ulcers at the center of burns. Acute inflammatory infiltrate was still evident with MEBO-treated burns (as compared to HS). Although MEBO-treated burns were more improved with a smaller lesion than that of the untreated groups, they did not completely heal and cysts (c) were detected under the regenerated epidermis due to exudate collection (Fig. 8B). Microscopic evaluation demonstrated that HS-treated burns (after 12 days) were so perfectly healed with intact re-grown epidermis, which was difficult to distinguish from normal skin. Also, topical proliferation of hair follicles was pretty good as compared to control and MEBO-treated burns (Fig. 8C).

4. Discussion

Burn wound healing is a complex process, in which residual epithelial cells proliferate in an integrated manner to form an intact epidermis. The primary aim of treatment of burns is to prevent infection, then to promote proliferation of epithelial cells. So as a wound dressing, a balance must be obtained between antibacterial efficacy and cytotoxicity (Tshukudu et al., 2010), and a moist environment is most suited for epithelialization and wound healing (Subrahmanyam, 1998). According to the desirable properties of ideal dressing, a hydrogel sheet composed of chitosan, honey and gelatin is expected to have a good effect on burn treatment.

For the manufacture of the hydrogel dressings, gelatin is used as a gel matrix with the content of 20 wt% in various samples, so that the blends can easily be hydrogels at room temperature. Chitosan and honey are used for the functional materials of the composite gels. Because of high viscosity of chitosan solution and undiluted honey, which disadvantage to the compatibility of the components, the contents of chitosan and honey are controlled at relatively low levels, not more than 0.5 wt% for chitosan and 20 wt% for honey. All the samples exhibited uniform, smooth and homogeneous appearance. The prepared hydrogel HS showed a compact and uniform structure indicating a good compatibility between the components (Fig. 5a and b).

It is important for wound dressings to absorb exudate quickly. The swelling behaviors of the hydrogel samples were studied. As seen from Fig. 2, the results of ESW and SDP showed that the hydrogel samples swelled quickly in PBS within 5 min but the capacity of water uptake declined because of the presence of honey. In fact, swelling and degradation of the hydrogel samples concurred in water and got balance at some period of time. The increase of temperature accelerated the equilibrium remarkably, particularly for the samples with honey, which were sensitive to warm water. At the beginning of swelling, water uptake had an absolute advantage over degradation. At the same time the compact structure of the samples were broken down gradually and filled with water, which promoted the dissolution of honey into water accelerating degradation, thereof the inner porous structure was left (Fig. 5d and f). While degradation dominated, SDP decreased gradually until the sample collapsed.

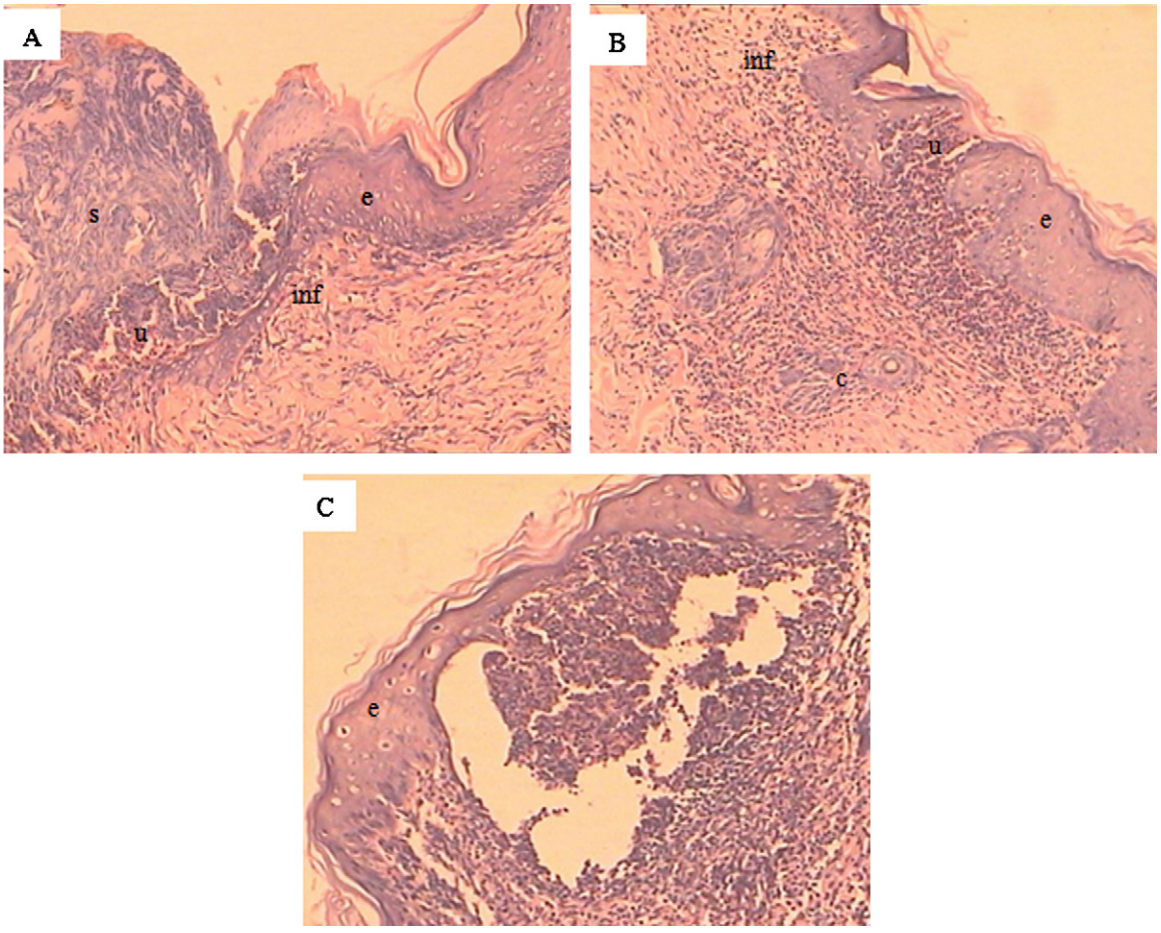

Fig. 8. Photomicrographs of burn wound tissues at day 12 post-burned: (A) untreated wound; (B) MEBO treated wound; (C) HS treated wound: s, scab; u, ulcer; inf, inflammatory cells; c, cyst; e, epidermis (100×).

The results of young's modulus of the samples ranging from 56 kPa to 112 kPa indicated that the hydrogels exhibited favorable mechanical properties for soft tissue repair (Yang et al., 2010). The presence of honey decreased the modulus of the corresponding hydrogels as compared with HS-4. HS-2 with 10 wt% honey showed the lowest modulus indicating the low amount of honey decreased the interaction of the polymers of chitosan and gelatin by scattering uniformly among them like plasticizer. While the content of honey increased to 20 wt%, e.g., HS and HS-3, the modulus of which rose again insignificantly, similar to those of the skin in various positions in humans.

The antimicrobial activity of the hydrogel dressing is more important. In order to obtain excellent antibacterial activity, however, the concentration of neat chitosan should be not less than 1.0 wt% (Zheng & Zhu, 2003) and 75 wt% for neat honey (Basualdo et al., 2007). In fact, when chitosan and honey were mixed together at significantly lower concentration 0.5 wt% and 20 wt% correspondingly, a positive synergistic effect was observed in terms of antibacterial activity of HS compared to HS-3 and HS-4. The HS hydrogel system functioned excellently in terms of antibacterial activity. Chitosan and gelatin have excellent miscibility to create a uniform structural semi-IPN hydrogel network due to the interactions between polymeric components such as electrostatic attraction, covalent unions, hydrogen bonding and dipole between others (Sionkowska, Wisniewski, Skopinska, Kennedy, & Wess, 2004; Yang et al., 2010). The amount of free amido has influence over the antibacterial properties of chitosan. Besides, the pH value of the hydrogel HS is about 5.5, at which all the bacteria, especially the Gram masculine bacteria, comprise negative charge. Hence the bacteria are easy to be captured by the hydrogel because of its presence of protonated positive ion, and then lose the physiological functions of reproduction and bioactivity (Deng, He, Zhao, Yang, & Liu, 2007). The role of honey is mainly due to chemical action on the cell. Hydrogen peroxide, volatiles, organic acids, flavonoids, beeswax, nectar, pollen and propolis are important chemical factors that provide antibacterial properties to honey. The physical characteristics of honey such as high osmolarity and acidity also contribute to its antibacterial activity (Basualdo et al., 2007). Additionally, the organic acids in honey increase the positive charge on chitosan amido ($-NH_2$) groups by protonation, improving the ability of adsorption onto cells (Liu, Wu, & Wang, 2010). Thus the multiple physicochemical functions of the HS system greatly enhanced antibacterial activity. According to the inhibition rates to *S. aureus* and *E. coli* of HS retaining 100% in 72 h, it suggested that the hydrogel system is stable enough for use.

Overall, the discussions above indicate that the hydrogel sheet HS is most optimal for dressing. HS is most excellent in the antibacterial activity, and soft suitable for skin repair. Being a hydrogel, HS has a weak point in water uptake, which limits HS to use as a dressing on wounds of less exduate.

In the case of burn wound healing, it is apparent that HS has a significant effect on burn wound contraction compared to others (Fig. 6). The micrographs also showed that HS-treated burns recovered with intact re-epidermis superior to that obtained with MEBO ointment (Fig. 8). The functional characteristic of the materials and the microenvironment HS created contribute to the wound healing.

Chitosan possesses favorable characteristics for promoting physiologically ordered dermal regeneration during wound healing. The main biochemical effects of chitosan are fibroblast activation, cytokine production, giant cell migration and stimulation of type IV collagen synthesis (Muzzarelli, 2009). It enhances production of extracellular matrix due to the higher hydrophilicity and degradation properties. During the wound healing process, chitosan gradually depolymerizes to release N-acetyl-β-D-glucosamine, which initiates fibroblast proliferation and helps in ordered collagen deposition and stimulates increased level of natural hyaluronic acid synthesis at the wound site (Jayakumar et al., 2011). Similarly, honey may play a positive role in modulating wound healing when incorporated into hydrogel matrix. It hastens wound healing by accelerating wound contractions (Yusof et al., 2007). In general, it results in a rapid clearance of infections, rapid debridement of wounds, rapid suppression of inflammation, minimization of scarring, and stimulation of angiogenesis as well as tissue granulation and epithelium growth (Basualdo et al., 2007). Moreover, gelatin could take part in the movement, differentiation and multiplication of cell (Deng et al., 2007), and endow hybrid hydrogel with favorable mechanical properties for soft tissue repair compared with pure gelatin and chitosan hydrogel (Yang et al., 2010).

A problem is that there are much more inflammatory cells at the HS-treated burn (Fig. 8C). It might be attributed to the presence of 0.5% acetic acid in HS, which has low toxicity, just acting as the solvent of chitosan. Some related literature about chitosan-based wound dressings also showed the presence of acetic acid (Alsarra, 2009; Ong, Wu, Moochhala, Tan, & Lu, 2008; Zhang et al., 2008). Considering the advantage of HS, we can assert that acetic acid has little effect on formulation stability and wound healing of HS. The result that wounds treated with MEBO had cysts left at the burn site due to the collection of exudate compared to HS indicated that HS has more powerful ability of absorption than MEBO. So the multiple effects of the HS system contributed to the excellent healing of burns.

5. Conclusions

In conclusion, a new dressing in the form of a composite hydrogel sheet produced from chitosan, honey and gelatin has been developed, which had powerful antibacterial efficacy to *S. aureus* and *E. coli*, and significantly promoted burns healing. Further investigation will more precisely delineate the mechanisms behind the improved antibacterial activity of HS, and define the types of wounds that can be treated successfully.

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