

## Research Article

# Chitosan-Alginate Sponge: Preparation and Application in Curcumin Delivery for Dermal Wound Healing in Rat

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A biodegradable sponge, composed of chitosan (CS) and sodium alginate (SA), was successfully obtained in this work. The sponge was ethereal and pliable. The chemical structure and morphology of the sponges was characterized by FTIR and SEM. The swelling ability, in vitro drug release and degradation behaviors, and an in vivo animal test were employed to confirm the applicability of this sponge as a wound dressing material. As the chitosan content in the sponge decreased, the swelling ability decreased. All types of the sponges exhibited biodegradable properties. The release of curcumin from the sponges could be controlled by the crosslinking degree. Curcumin could be released from the sponges in an extended period for up to 20 days. An in vivo animal test using SD rat showed that sponge had better effect than cotton gauze, and adding curcumin into the sponge enhanced the therapeutic healing effect.

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## 1. Introduction

The process of wound healing is a set of coordinated responses to tissue injury that results in tissue contraction, closure, and restoration. The longer it takes for spontaneous wound healing, the worse the outcome usually is, with increasing likelihood of developing hypertrophic scarring and unsightly alterations in pigmentation. Moreover, under unfavorable conditions, the self-perpetuating inflammatory cascade may result in increasing tissue destruction and necrosis rather than healing [1]. The healing process can be thought of as occurring in five phases, namely, inflammation leading to haemostasis and clot formation, fibroplasia and neovascularization, formation of granulation tissue, reepithelialization, and finally the formation of new extracellular matrix and tissue remodeling [2]. Inflammation is closely associated with the release of inflammatory mediators including Reactive Oxygen Species (ROS), Reactive Nitrogen Species (RNS), along with their derivatives. These radicals will result in oxidative stress leading to lipid peroxidation and ultimately cause local and distant pathophysiological effects.

However, topical applications of compounds with antioxidative properties using proper materials and antioxidants will be useful against oxidative damage and be helpful to the healing of the wound.

Chitosan and alginate are well known for accelerating the healing of wounds in humans [3, 4]. Chitosan (CS), a natural cationic polymer, is biologically renewable, biodegradable, biocompatible, nonantigenic, nontoxic, and biofunctional, and it can accelerate the wound healing process enhancing the functions of inflammatory cells, macrophages, and fibroblasts [3, 5, 6]. Alginate, an anionic polymer, also is biocompatible, hydrophilic, and biodegradable under normal physiological conditions. It is able to maintain a physiologically moist microenvironment that promotes healing and the formation of granulation tissue and achieves hemostasis [7]. In this study, we used these two kinds of polymer to form complexes through chemical binding. After lyophilization, which creates the porous structure, the sponge formed to act as a topical applications matrix.

In this work, curcumin as a multifunctional agent was incorporated into the chitosan and alginate sponge

(CA sponge) to deter wound infection. Curcumin (diferuloylmethane) is an orange-yellow component of turmeric (*Curcuma longa*), a spice often found in curry powder [8]. It has been shown to exhibit numerous activities. Various preclinical cell culture and animal studies suggest that curcumin has potential because of its pharmacological properties including anti-inflammatory [9, 10], antimicrobial [11], antiviral [12, 13], anticancer [14], antioxidant [15–17], chemosensitizer [18, 19], radiosensitizer [20, 21], and wound healing activities [22–24]. Topical applications of curcumin with its properties of anti-inflammatory antioxidant (free radical scavenging activity), induction of detoxification enzymes, and providing protection against degenerative disease in patients have shown to improve significantly wound healing and protect tissues from oxidative damage [24].

## 2. Materials and Methods

**2.1. Materials.** Chitosan (Mw 300,000 and degree of deacetylation of 92%), sodium alginate (Mw 300,000), was purchased from Chengdu Ronghai Bio-Tech Development, China.

Curcumin was purchased from Sigma-Aldrich Chemical Co. (USA). All other reagents and solvents used were of reagent grade.

**2.2. Preparation of Chitosan-Alginate Sponge (CA sponge).** Chitosan solution (1.0% w/v) was prepared by dissolving chitosan powder (0.2 g) in 20 ml of deionized water containing acetic acid (1.0% by weight) at room temperature. Alginate solution (1.0% by weight) was prepared by dissolving sodium alginate powder (0.2 g) in 20 ml of deionized water at room temperature. The dissolved chitosan solution was then added into the alginate solution (the chitosan-alginate blend ratios by weight were 3 : 1, 1 : 1 and 1 : 3) and blended with an homogenizer (IKA T10, Germany) until an opaque aqueous solution was obtained. The solution was centrifugated to remove the trapped air bubbles. Then the air bubble-free solution was poured into the wells of a 24-well plate (well size: 17 mm diameter, 20 mm height) or a 6-well plate (well size: 36 mm diameter, 20 mm height), frozen overnight at  $-40^{\circ}\text{C}$ , and then lyophilized at  $-35^{\circ}\text{C}$  for 24 hours in a freeze dryer (VIRTIS Advantage wizard 2.0, USA).

**2.3. Preparation of Curcumin-Loaded CA Sponge.** Curcumin was accurately weighed and dissolved in absolute ethanol. The curcumin-ethanol solution was mixed with chitosan solution. The mixed curcumin/chitosan solution was added into the alginate solution and blended, centrifuged, frozen, and lyophilized as described above.

**2.4. Fourier Transform Infrared Spectroscopy (FTIR).** The characteristic absorption of chitosan-alginate interpolyelectrolyte complex, which ranged from 400 to  $4000\text{ cm}^{-1}$ , was recorded on a NICOLET 200SXV Infrared Spectrophotometer (USA).

**2.5. Morphology (SEM).** The morphology of chitosan-alginate sponge was investigated by scanning electron

microscopy (SEM, H-6009IV, Hitachi, Japan). Specimens were cut and coated with gold. The sponge microstructures were investigated by geometrical measurement on the scanning electron micrographs.

**2.6. Swelling Ability Study of Sponges.** The swelling ability of chitosan-alginate sponges was determined by incubating the CA sponges in pH7.4 of phosphate buffer saline (PBS) at room temperature. A known weight of CA sponge was placed in the media. The wet weight of the sponge was determined at required period of time by first blotting the sponge with filter paper to remove adsorbed water on the surface, then weighed immediately on an electronic balance. The percentage water adsorption of chitosan-alginate sponges in the media was calculated as follows:

$$E_{sw} = \left[ \frac{(W_e - W_0)}{W_0} \right] \times 100, \quad (1)$$

where  $E_{sw}$  is the percentage water adsorption of chitosan-alginate sponges at equilibrium.  $W_e$  denotes the weight of the chitosan-alginate sponges at equilibrium water adsorption, and  $W_0$  is the initial weight of the chitosan-alginate sponges. Each experiment was repeated 3 times, and the average value was taken as the percentage water adsorption.

**2.7. In Vitro Degradation.** The chitosan-alginate sponges were incubated at pH 7.4 in phosphate-buffered saline (PBS) with 500–1000 U/C.C. of lysozyme concentration in 6-well plate and kept at  $37^{\circ}\text{C}$ . At required period of time, the sponges were taken out, washed with deionized water, frozen, and lyophilized. The weights of the sponges were weight and recorded:

$$\text{Weight loss (\%)} = \left[ \frac{(W_0 - W_t)}{W_0} \right] \times 100. \quad (2)$$

**2.8. Drug Loading and Release.** Curcumin-incorporated CA sponge was prepared by first adding curcumin ethanol solution (final concentration was 1 mg/ml in chitosan-alginate solution) to chitosan solution under consequently stirring. Then the mixed solution was added into alginate solution, blended, centrifuged, frozen, and lyophilized as described above.

Drug release experiments using 17 mm-in-diameter curcumin-incorporated CA sponges were conducted in 6 centrifuge tubes with 10 ml phosphate-buffer solution (pH 4) within a shaker at  $37^{\circ}\text{C}$ . All the supernatants were pipetted out periodically and replaced with equivalent volume of fresh phosphate-buffer solution. The concentration of curcumin was measured using HPLC (Waters, USA) at 420 nm.

**2.9. Wound Healing Test.** The Sprague-Dawley (SD) rats (160–180 g, 6 weeks) were used in this wound healing test. The animals were anaesthetized by using chloral hydrate prior to the test. The dorsal hair of the rats was removed. Full-thickness wound of  $1.5 \times 1.5\text{ cm}^2$  was excised from the back of the rats. Each wound was covered with an equal size of drug loaded sponge, or blank sponge, or cotton gauge for comparison. On top of the wound dressings, a piece of

Tegaderm (3M, USA) was applied. Treated rats were placed in individual cages, and the healing wounds were observed on the 0th, 4th, and 12th days using a digital camera.

Skin wound tissue of rat was excised, fixed with 10% formalin, and stained with hematoxylin-eosin (H&E) reagent for histological observations.

### 3. Results and Discussion

**3.1. Preparation of Chitosan-Alginate Sponge and Curcumin-Incorporated Chitosan-Alginate Sponge.** The chitosan-alginate sponges were successfully prepared in this work and were shown in Figure 1(a). In Figure 1(b), the sponge was bent with forceps, which showed that this sponge is flexible.

**3.2. Fourier Transform Infrared Spectroscopy (FTIR).** To examine the chemical structures of various CA sponges prepared under different interpolyelectrolyte complex conditions, the CA sponges were examined by FTIR spectra. Figure 2 shows the FTIR spectrum from different chitosan/alginate blend ratios. The characteristic peak of chitosan were seen at  $1650$  and  $1590\text{cm}^{-1}$  corresponding to amide and amino group. The alginate spectrum shows characteristic band of carbonyl (C=O) band ( $1640$  and  $1424\text{cm}^{-1}$ ). The characteristic absorption of  $-\text{NH}_3^+$  shifted to  $1622\text{cm}^{-1}$  in spectrums of three kinds of sponges. The intensity of the characteristic peak order was  $\text{C2A2} > \text{C3A1} > \text{C1A3}$ ; this indicated that the degree of ionic interaction between the negatively charged carboxylic ion group of alginate and the positively charged amino group of chitosan in three kinds of sponges was different.

**3.3. Morphology Study.** The cross-section morphology of the sponges is shown in Figure 3. Depending on the composition, there were clear differences in the appearance of the fibrillar structure of the sponges. As shown in Figures 3(a) and 3(b), the pure alginate and chitosan showed a relatively regular network. The pore sizes of the chitosan and alginate are of approximately  $200$  and  $100\mu\text{m}$ , respectively. The mixture, however, as shown in Figures 3(c) and 3(d), showed a much more irregular morphology. These differences may be explained by that the mixed systems may have interacted prior to the freezing and therefore would have already formed a random fibrillar network. However, the single component system would have remained in solution; hence the pore size is determined by ice crystal formation during the freezing process. This may be reasonably expected to be homogeneous throughout the sample, leading to a more regular pore size and fibrillar structure [6].

**3.4. Water Uptake Ability.** The water uptake ability of the chitosan-alginate sponge was listed in Table 1. All the sponges absorbed water within 30 seconds and were saturated within 1 minute. The sponges with microporous structures seem to show the difference in the water adsorption depending on the porous structure, which is attributed to the water retained within pores. From Table 1, the water uptake of sponges decreased as we increased the ratio of alginate to chitosan up to 3. This result should depend on the pore structure. The C3A1 sponge can

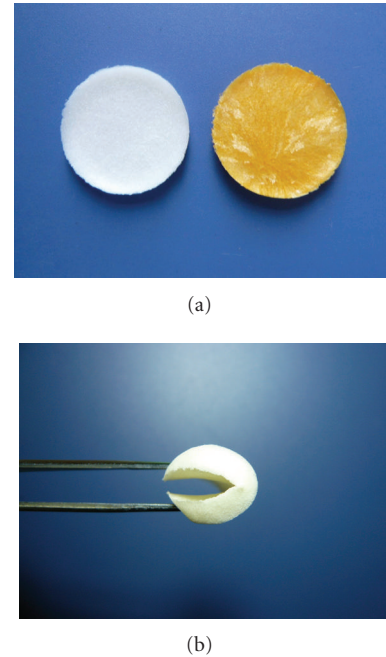


FIGURE 1: Photograph of chitosan-alginate sponge (C2A2). (a) Appearance (left, pure sponge; right, sponge with curcumin), (b) sponge showing the pliability of the freeze dried material.

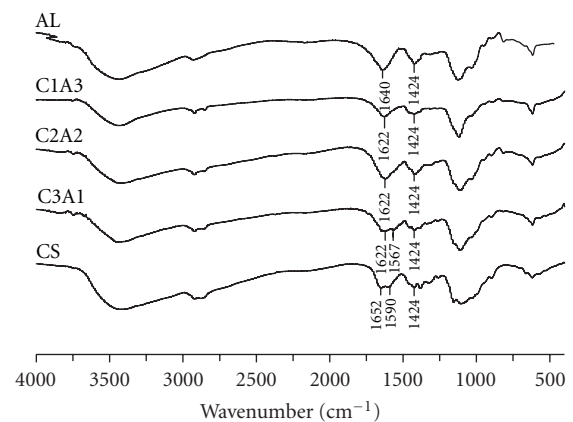


FIGURE 2: FTIR spectra of various sponges.

TABLE 1: Water uptake ability of chitosan-alginate sponge.

Sponge type	Mixing ratios		Water uptake ability (%)
	Chitosan	Alginate	
C3A1	3	1	4290
C2A2	2	2	3806
C1A3	1	3	1218

hold water in each pore surrounding by cell walls and the water uptake ability reaches more than 4000%. The C2A2 sponge decreased to 3806% because the fibrous networks contained in the sponge attenuate the ability of the water retained within pores. On the other hand, the C1A3 sponge gave a smaller value of about 1000%, in which the

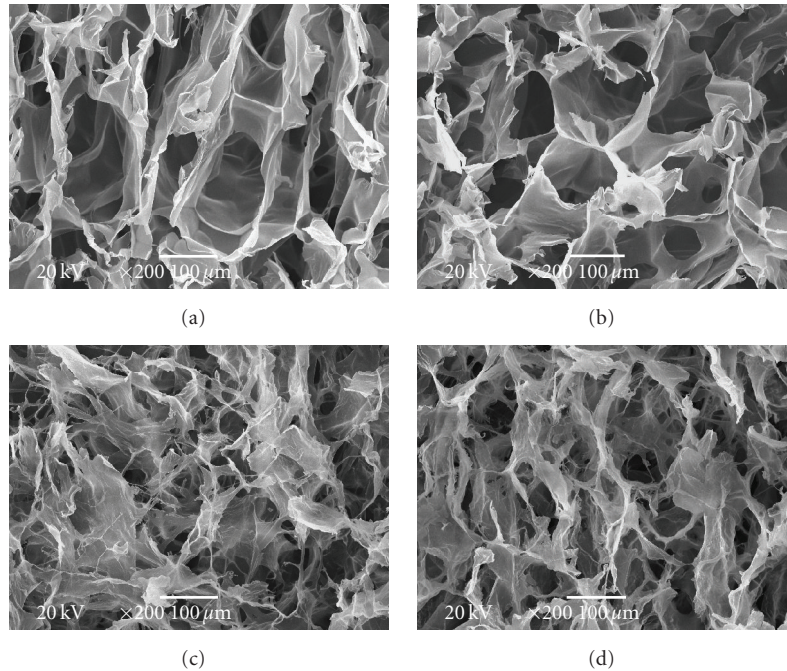


FIGURE 3: SEM micrographs of chitosan-alginate sponges: (a) alginate alone, (b) chitosan alone, (c) C2A2, and (d) Curcumin-C2A2.

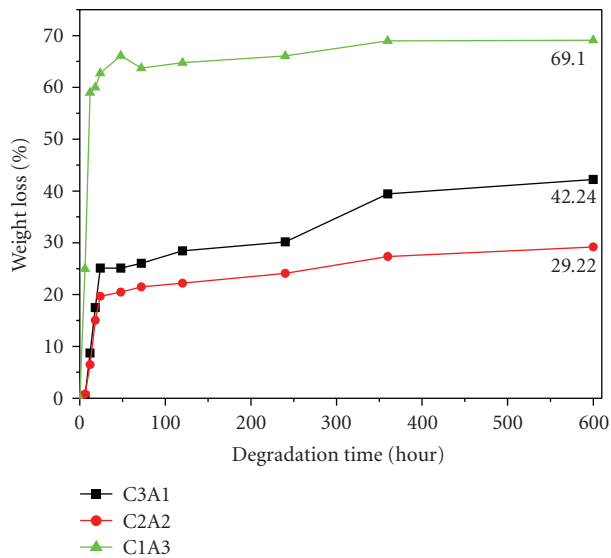


FIGURE 4: In vitro degradation of CA sponge in PBS lysozyme solution.

pores are surrounded by the thin and fragile walls. The walls might be comprised by a part of the unreacted alginate; when the sponge was dropped into the water, the unreacted alginate swelled and dissolved making the pore size increase and therefore could not sustain much water within their network structure. Another reason that might decrease the water uptake ability of the sponge is the sealed-in cells. Water can not get into the sealed-in cells resulting in a decrease in the volume of water that can be held in the sponges.

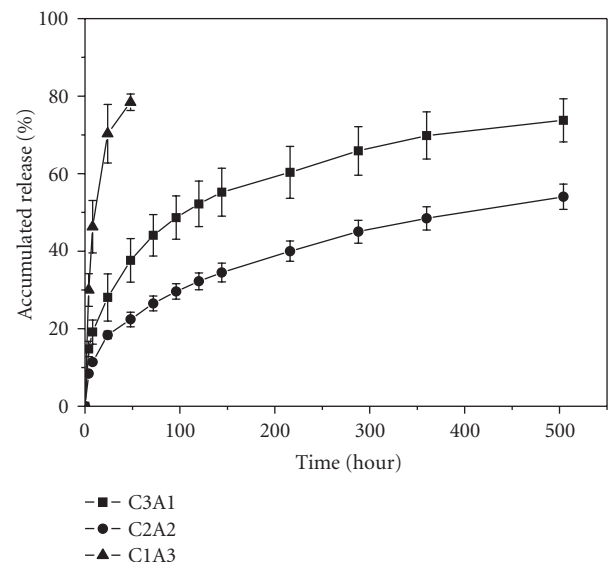


FIGURE 5: The in vitro release behaviors of curcumin from chitosan-alginate sponges.

**3.5. In Vitro Degradation.** To examine stability during a few days of contact with the wound, we examined stable property of CA sponges. The percentage of weight loss of crosslinked sponges as a function of degradation time is presented in Figure 4. The weight loss for CS sponges ranges from 25% to 70%. The sponges prepared with the mixing ratio of chitosan to alginate of 1 : 3 showed 1.5–3 times higher weight loss than that of 3 : 1 and 2 : 2. The order of the weight loss was C1A3>C3A1>C2A2. The result indicated that the C2A2



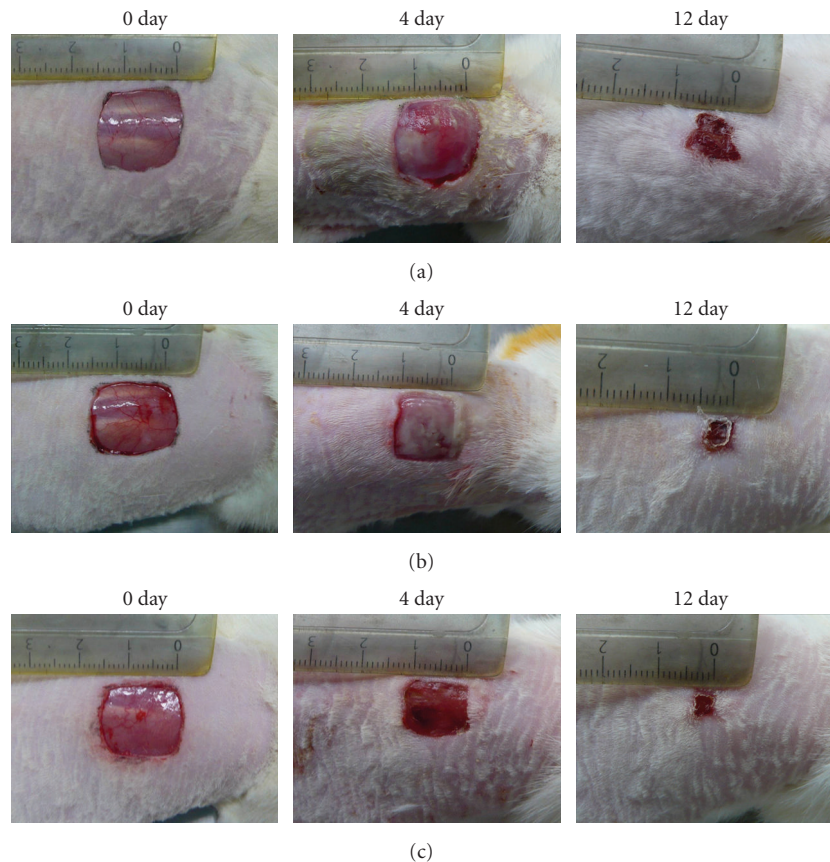


FIGURE 6: Photographs of macroscopic appearance of wound repair covered with (a) cotton gauze as control, (b) C2A2 without curcumin, and (c) C2A2 with curcumin, at day 0, day 4, and day 12, respectively.

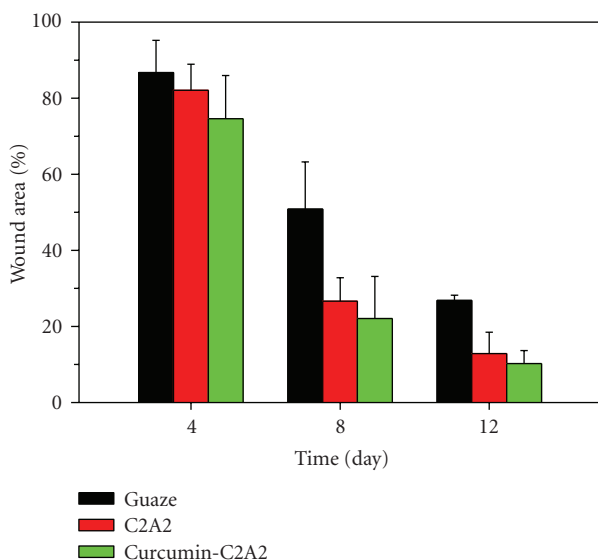


FIGURE 7: Total wound area of skin over time as a percentage of original wound size.

sponge was more stable than the C3A1 and C1A3 sponges, probably because crosslinking degree of C2A2 sponge was stronger than the others, then was C3A1, and C1A3 was the last one.

**3.6. Drug Loading and Release.** The release of curcumin from sponge was investigated to evaluate its sustained ability. Figure 5 shows the drug release behaviors of chitosan-alginate sponges loaded with curcumin which was a hydrophobic drug. In 20 days, about 50% and 70% of the drug were released, respectively, from C2A2 and C3A1 sponge. While 80% of the drug within the C1A3 sponge had been released in 3 days. This indicated that the C2A2 sponge has a better sustained release property than the C3A1 and C1A3 sponges. We thought that it can be attributed to the interaction of amine groups on chitosan with carboxyl groups on alginate. From the result of FTIR, we know that the order of ionic interaction degree of three kinds of sponges was C2A2>C3A1>C1A3. So the trapped drug in the C2A2 sponge released from the network structure was the slowest, and that of C1A3 was the fastest.

**3.7. Wound Healing Test.** As the results of item 3.4–3.6, we chose sponge C2A2 as our wound dressing in wound healing test. In wound testing, full-thickness wounds were made on the back of each rat (Figure 6). CA sponges with or without drug were used to study wound healing behavior. As a control, the wound was covered with cotton gauze.

On the 4th postoperative day the cotton gauze adhered to wound surface and removal of it resulted in the less of tissue at the wound surface. The C2A2 and C2A2-Curcumin

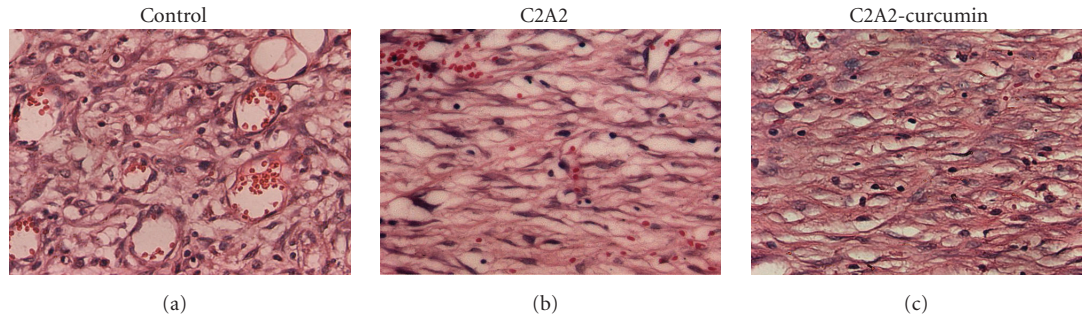


FIGURE 8: The H&E stained sections of twelve-day postwounding (40 $\times$ ).

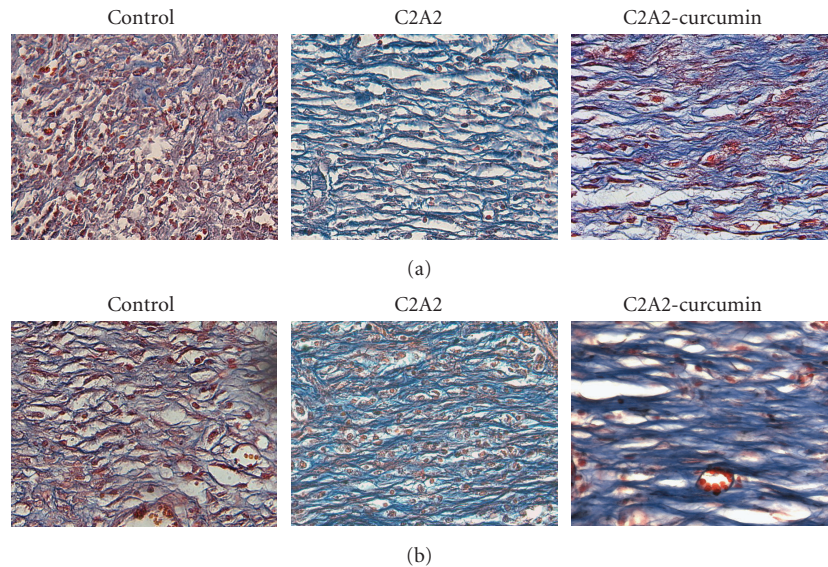


FIGURE 9: Masson's Trichrome staining for collagen: (a) eight-day postwounding; (b) twelve-day postwounding (40 $\times$ ).

sponge also adhered to the wound surface and absorbed the bleed and exudation at the wound site. We can see slight bleeding and inflammation at the wound site when the C2A2 sponge peeled away, while the underlying granulation tissue was formed in the case of C2A2-Curcumin sponge.

The wound on the 12th day was more contractive than the wound observed on the 4th postoperative day; the healing of the C2A2 and C2A2-Curcumin sponges treated wound was obviously faster than gauze-treated wound.

Figure 7 shows the contraction of the wound treated with different means. By observing the wound area at preset time, the reduction in wound defect area was calculated. From Figure 7, we could see a little difference between wound area of three groups on the 4th postoperative. But by the 8th day, the C2A2 and C2A2-Curcumin sponge-treated wounds healed more quickly than the cotton gauze-treated wounds. And the wound areas are almost half of the cotton gauze treated wound area. On the 12th day, the C2A2 and C2A2-Curcumin sponge-treated wounds contracted, respectively, 88% and 90%, whereas the control group contracted 74%. Little difference was seen between the C2A2 and C2A2-Curcumin sponge-treated wounds. This indicates that the

chitosan-alginate sponges with or without curcumin are all suitable for a better wound healing effect, and the wound healing process seemed to mainly depend on the use of the property of sponge itself. It suggested that the prepared CA sponge could be a good material to be employed as wound dressing.

**3.8. Histological Examination.** Figure 8 exhibits the histological results of three different kinds of wound dressings applied on the dorsal skin wound of SD rat. On the 12th day, the dermis is still under the process of remodeling. The gauze-treated wounds showed immature granulation tissue with numerous inflammatory cells and congested vessels (Figure 8(a)). However, the granulation tissue and collagen alignment (Figures 8(a) and 8(b)) were more advanced in the C2A2 sponge and C2A2-curcumin sponge-treated wounds as compared to the gauze-treated wounds. And the vessel numbers were smaller in the sponges-treated wound. These two kinds of sponges-treated wounds demonstrated compact and well-aligned collagen, showing that the wounds were under a better healing environment.

The extent of collagen deposition in the wounds was examined by Masson's Trichrome staining. From Figure 9,

we could see that gauze-treated wound had a loose reticular arrangement of collagen, the space between the collagen was relatively large, whereas collagen was relatively compact, the bundles of collagen showed a better ordered arrangement in the C2A2 sponge-treated wounds, and the collagen content was greater than the former. The C2A2-Curcumin sponge-treated wound also had a compact and well-aligned arrangement of collagen, and the bundles of collagen were thicker than both of the gauze and the C2A2 sponge-treated wounds. This study showed that the C2A2 sponge can promote the formation of the collagen, thus accelerate the healing of wound. The use of curcumin seemed to have a certain promoting effect on wound healing according to the collagen content and arrangement.

#### 4. Conclusions

In this work, the crosslinked sponges based on chitosan and alginate were successfully prepared at the various conditions of mixing ratios 3 : 1, 2 : 2, 1 : 3, and water uptake ability ranging between 1000% and 4300%. Based on the results of drug release of curcumin, we found that the C2A2 sponges have a sustained release behavior for up to 20 days. This shows that the C2A2 sponge could be a good drug support to be employed for sustained release. Based on the preliminary histological results on the 12th postoperative days, the C2A2 and C2A2-Curcumin sponges showed a better wound healing effect in view of a rapid contraction of wound, compared with the gauze-treated wounds. Although no significant difference of the reduction in wound defect area was seen between the C2A2 and C2A2-Curcumin sponges-treated wounds, a greater content and a better arrangement of collagen in C2A2-Curcumin sponges-treated wounds showed that adding curcumin into the sponge has a certain promoting effect on wound healing.

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