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Using absorbable chitosan hemostatic sponges as a promising surgical dressing

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ABSTRACT

As absorbable hemostatic dressings, chitosan with a deacetylation degree of 40% (CS-40) and 73% (CS-73) have been fabricated into sponges via a modified method. The hemostatic, biocompatible and biodegradable properties were evaluated through in vivo assays. In a hepatic hemorrhage model, the chitosan sponges, with excellent blood compatibility, achieved less blood loss than the gelation sponge (GS). In addition, CS-40 showed better hemostatic capability and biodegradability than CS-73. After implantation, a histological analysis indicated that CS-40 exhibited the best biodegradability, tissue regeneration and least tissue adhesion. By contrasting CS-40 and CS-73, the deacetylation degree is confirmed to be a key factor for the hemostatic effect, biodegradability, biocompatibility and tissue regeneration. Our overall results demonstrated the potential application of CS-40 for use in absorbable hemostatic dressings.

1. Introduction

Uncontrolled hemorrhaging is the main cause of death due to military trauma and also in civilian settings. Absorbable hemostatic agents have been widely developed to avoid reoperation. For use as a surgical treatment, an ideal absorbable hemostatic dressing must be safe, rapidly effective, nonantigenic and show biocompatibility [1,2].

Nowadays, a number of absorbable hemostatic dressings have been produced including porcine or bovine gelatin [3], collagen, regenerated oxidized cellulose [4] and so on. However, none of these dressings can claim to be the ideal solution, because of some notable adverse effects such as toxicity, potential infections and virus transfusion [5].

As a kind of natural polymer, chitosan (CS) has been widely utilized for hemostatic applications due to its excellent biocompatibility [6–10], biodegradable properties [8,11–13], along with its hemostatic [14,15] and antimicrobial activities [16–18]. A lot of chitosan-based dressings have been produced such as Hemcon and Celox. As the main component of these dressings, chitosan acetate can improve the hemostatic efficacy by enriching the hemocytes. But the acid within the dressings can lead to inflammatory reactions and poor biodegradation in vivo [19,20]. Therefore, the objective of this study is to obtain a novel chitosan dressing with both high hemostatic efficacy and good biodegradability.

It has been proved that the degree of deacetylation (DD) is the key factor for hemostatic properties and biodegradability. Some studies found that a lower DD lead to better hemostatic efficacy [21,22]. In a subcutaneously implanted model [23], the CS with lower DD showed better biodegradability. Therefore, we hypothesize that chitosan with low DD may result in both a faster hemostatic effect and biodegradation. For comparison, chitosan with medium DD was also prepared and measured.

A quick and easy method called the "mimicking paper-making method" [24] has been reported for the preparation of porous cellulose membrane. Chitosan gel can also be prepared by the above method and fabricated into sponge by freeze-drying just one time. Compared with traditional methods, the modified method can save time by reducing the time required for freeze-drying and making the sponge surface coarser to absorb more hemocytes [25].

In conclusion, chitosan with relatively low DD was fabricated into sponges by a modified method for use as surgical dressings. As a widely used absorbable hemostatic dressing, a gelatin sponge was used as a control group. The DD, morphology, porosity, swelling

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ability and degradation behavior in vitro were investigated by various physicochemical methods. The hemostatic, biocompatible and biodegradable properties were evaluated through in vivo assays.

2. Materials and Methods

2.1. Materials

Chitin and chitosan (Mv of 5.01×10^5, DD of 93%) were purchased, respectively, from the Golden-Shell Biochemical Co., Ltd (Zhejiang, China) and Qingdao Hecreat Bio-tech Co., Ltd (Shandong, China). Absorbable gelatin sponges were commercially obtained. All other reagents were of analytical grade and used as-received unless otherwise noted.

2.2. Synthesis and characterization of chitosan with modified DD

According to our previous research [26], chitosan samples with different DD (from low to high) can be successfully synthesized. The chitosan with low DD was synthesized by dissolving raw chitosan (2 g) into a 60 ml aqueous acetic acid solution (2%, v/v). Then 80 ml of methanol was added and stirred for 10 min. Acetic anhydride (0.55 g) was then added and stirred for 15 min. After standing for 2 h, the solution was filtered and the filtrate was precipitated with 1 M KOH solution dissolved in a ethanol–water mixture (1:1, v/v). The precipitate was washed to neutral with tri-distilled water and dried at 60 °C under vacuum. The chitosan with medium DD was synthesized by dispersing chitin (10 g) in an H_2O_2 aqueous solution (7.6%, v/v) at 50 °C for 1 h. Then a 300 ml of NaOH aqueous solution (0.5 g/ml) was added and stirred for 1 h at 90 °C. The chitosan powder was washed to neutral and dried at 60 °C under vacuum.

The DD of the two chitosan samples was measured by the potentiometric titrations method, ^{1}H NMR and FT-IR spectra.

2.3. Fabrication of chitosan sponges

To fabricate the chitosan sponges, two chitosan samples (0.5 g) were, respectively, dissolved in 90 ml of acetic acid aqueous solution (1%, v/v) and then a 12.5 ml NaOH solution (2 mg/ml) was added to obtain a homogeneous gel suspension. The chitosan gel was collected by centrifugation and washed to neutral by tri-distilled water. Then a 50 ml of glycerol solution (10 mg/ml) was added and stirred for 30 min. Through filtration, a chitosan gel was obtained again and fabricated into sponges by freeze-drying at −50 °C overnight. The finished products with a thickness of 1.50 ± 0.05 mm were sealed and γ-irradiated with a dosage of 25 kGy.

2.4. Measurement of morphology and pore structure

The morphology of sponges was observed by a JSM-5510 LV scanning electron microscope (SEM, JEOL, Japan). The average pore diameter, pore size distribution and porosity were studied by using a mercury porosimetry (Micromeritics' AutoPore IV 9500 Series).

2.5. Swelling measurement

The dry dressings (weight m_d and exterior volume V_d) were placed into tri-distilled water at 37 °C for 24 h [25]. The wet weight (m_w) and exterior volume (V_w) of the swollen dressings were measured immediately after gently blotting them with filter paper to remove any surface liquid. The water absorption capacity of the dressings were calculated using the formula: $W_{ab} = (m_w - m_d)/m_d$. The swelling ratio was calculated according to the formula: swelling ratio (%) $=(V_w - V_d)/V_d \times 100\%$.

2.6. In vitro lysozyme degradation test

The lysozyme degradation test was carried out as described in reference [27]. The chitosan samples were equally weighed (0.2 g) and immersed in 30 ml of phosphate-buffered saline (0.1 mol/l, pH 7.4) with lysozyme (70,000 U/mg) and NaN_3 (0.5 mg/ml), then incubated at 37 °C for 28 days. The sponges were removed after 7, 14, 21 and 28 days. The sponges were washed with tri-distilled water to remove any ions adsorbed on the surface and then freeze-dried. The dry weight was noted as W_d. The degradation of the sponges was calculated using the formula: Weight remaining percentage (%) $= (W_d/0.2) \times 100\%$. After centrifugation at 5000 rpm for 10 min, the pellucid supernatant was collected and then treated based on the modified Schales method [27]. The absorbance of the solution at 420 nm was recorded by a UV–vis spectrophotometer (Hitachi U-1000, Japan). The content of the reducing sugar could be gained from the calibration curve of the D-glucosamine (Sinopharm Chemical Reagent Co. Ltd).

2.7. In vivo evaluation of hemostatic effect

Thirty Sprague–Dawley (SD) rats (male, 220–250 g) were randomly divided into three groups, with each group containing 10 rats ($n = 10$). For liver injuries, rats were anesthetized with pentobarbital sodium and through using an abdomen section, the livers were exposed. According to reference [28], a wound measuring approximately $1 \times 1 \times 0.2$ cm^3 was made on the left lateral hepatic lobe. After 10 s of bleeding, the sponges were applied to the site of the wound and were manually pressed for 1 min, then released. If the hemorrhage still occurred, a second piece of dressing was applied and held with pressure as described above. The same procedures were repeated until the hemorrhaging ceased. Once hemostasis was confirmed, a piece of hemostatic dressing with certain weight was pushed into the defective site and covered with a right liver lobe to avoid potential adhesion to the abdominal wall or other organs. Then the abdomen was closed and the rats were allowed to recover from anesthesia. The hemostatic time was recorded and dressings used were weighed to calculate total blood loss. The survival rate during the first 4 h was calculated in case of chronic bleeding. Posttreatment rats were housed individually and allowed to freely have water and their normal diet. All animals were observed twice a day for the first 5 days and once a day for the rest of the time. The general states of health, such as activity, diet and symptoms of infection were recorded.

2.8. In vivo evaluation of biodegradability and biocompatibility

The posttreatment rats were used to evaluate the biodegradability and biocompatibility of implanted sponges after 1, 4 and 8 weeks. At each time point, three rats of each group were weighed and euthanized with pentobarbital sodium. Then fresh blood was collected from the orbital vein for analysis of blood compatibility. The implants with surrounding tissues and lung tissues were retrieved for macroscopic and histopathological evaluation. The tissues and implants were fixed with 10% formalin. After the gradient ethanol dehydrated, the samples were paraffin embedded, sectioned, stained with hematoxylin and eosin (H&E) and Masson's trichrome for microscopic examination.