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Effects of PVA sponge containing chitooligosaccharide in the early stage of wound healing

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Poly(vinyl alcohol) (PVA) sponges with different chitooligosaccharide (COS) content were prepared for wound-dressing application. The morphological structure of PVA sponges was observed by scanning electron microscopy. As the concentration of COS-loaded PVA sponge increased, the average pore size of sponge decreased and the release rate of COS from the sponge also slightly decreased. The accelerating effect of the COS-loaded PVA sponges on open wound healing in rats was investigated by macroscopic examination and measurement of wound area. The COS-loaded sponges were found to be very effective as a wound-healing accelerator in the early stage of wound healing. The wound treated with the COS-loaded PVA sponge was almost reepithelialized, granulation tissues in the wound were considerably replaced by fibrosis at 8 days after initial wounding. The COS-loaded PVA sponge was considered to be a suitable wound-healing formulation due to its easy preparation and high effectiveness.

1. Introduction

Recently, much attention has been paid to chitosan as a functional biopolymer because it has several distinctive biomedical properties such as nontoxicity, biocompatibility, biodegradability, hemostatic activity, antimicrobial activity, and hydrophilicity [1–3]. The above biological activities exerted by chitosan are particularly useful for wound-dressing applications. Since chitin or chitosan has been used for wound healing as a folk remedy for a long time, there were numerous reports that it accelerated wound-healing in many clinical cases. However, chitin- or chitosan-based wound-healing products are still at the early stages of research.

It has been suggested that chitosan may be used to inhibit fibroplasia in wound healing and to promote tissue growth and differentiation in tissue culture [4]. Kim and Min [5] have developed a wound-covering material from polyelectrolyte complexes of chitosan with sulfonated chitosan. It was proposed that wound healing was accelerated by the oligomers of chitosan degraded by tissue enzymes and this material was found to be effective in regenerating the skin tissue in the area of the wound.

The oligomer of chitosan, chitooligosaccharide (COS), is known to be easily prepared by acidic or enzymatic partial hydrolysis of chitosan. It has been reported that lower oligomers of chitosan are water-soluble and biologically active, though their solubility and activities are dependent on molecular weight and degree of deacetylation [6, 7]. The oligomers of chitin or chitosan also have structural characteristics similar to glucosaminoglycans which are structural or physiologically active components of living tissues. Specific applications of chitin and chitosan oligomers include the search for hemostatics, immunostimulants, antitumor agents, enhancement of host–pathogen interaction in plants, drug carriers, chelating agents, and antimicrobial agents, and so on [8–10]. With respect to wound dressing, the COS was expected to be more effective wound-healing

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accelerator than chitosan because of a relatively fast and high interaction between the wound and water-soluble or water-swellable COS in a moist healing environment.

On the other hand, Poly(vinyl alcohol) (PVA) has several useful properties including nontoxicity, biocompatibility, high hydrophilicity, fiber- or film-forming ability, chemical and mechanical resistance. It has been widely commercialized or studied in the chemical and medical industries for the productions of fibers, films, coatings, cosmetics, pharmaceuticals, and so on. Moreover, blending with PVA has a great affinity for skin and extracellular matrix materials. Therefore, it was expected that COS-loaded PVA sponge might produce a highly elastic sponge membrane facilitating collagen synthesis of skin tissue [11].

In this study, we attempted to use the COS as a component of PVA sponge for wound dressing. The accelerating effect of COS on open wound healing in rats was investigated using the COS-loaded PVA sponge. For open wound healing, full-thickness skins in rectangles were removed on the backs of the rats and then the sponges were applied on the wounds. Macroscopic examination and measurement of wound area was performed.

2. Materials and methods

2.1. Materials

Chitooligosaccharide, which was kindly supplied from Hyosung Co. (Korea), has the compositions as follows: dimer 2.31, trimer 12.53, tetramer 15.11, pentamer 13.59, hexamer 8.86, heptamer 6.46, octamer 8.87, nanomer or higher 32.27 mol %. The degree of deacetylation (DD) of original chitosan was 87%. PVA (degree of polymerization, DP = 1770, degree of saponification, DS = 99%) was purchased from Aldrich Co. (USA). Rats for *in vivo* wound-healing test were purchased from the Jung-ang Animal Center (Korea). Other reagents were used without further purification.

2.2. Preparation of COS-loaded PVA sponges

The COS was dissolved in double distilled water at 20 °C for 3 h to prepare its 1, 2, 3% (w/v) solutions. PVA was dissolved in double distilled water at 95 °C for 1 h to prepare 1, 2, 3% (w/v) solutions, and its solution was slowly cooled down to room temperature. The two solutions were mixed at room temperature according to the predetermined ratios (PVA/COS = 100/0, 90/10, 80/20, 70/30, 50/50, w/w), and then stirred for 3 h. The mixed solution was cast on the polystyrene petri dish and frozen at − 80 °C for at least 8 h. The sponges were prepared by lyophilization of the frozen solution for 2 days. Sponge samples were sterilized with ethylene oxide gas before the wound-healing test.

2.3. Morphological observation of sponge

The morphological structure of PVA sponges prepared at different concentrations was examined by scanning electron microscopy (SEM, Hitachi S-2350). Also, the average pore size of sponges was measured by Image Analyzer (Scope Eye).

2.4. Antimicrobial activity of COS-loaded PVA sponge

AATCC Test Method 100-1993 was used for determining the antimicrobial activity of COS-loaded sponges. In this method a gram-positive bacterium, *Staphylococus aureus* (ATCC 6538), was used. The results were expressed as reduction in bacteria (%).

2.5. *In vitro* release behavior of COS

The COS-loaded sponge (0.1 g) was placed in the sample bottles containing 10 ml of phosphate buffered saline (PBS, pH 7.4) and eluted in a shaking water bath at 37 °C. The amount of COS eluted was determined from the calibration curve, using an optical density at 280 nm by UV spectrophotometer (HP 8452A).

2.6. Open wound-healing test

Twenty rats weighing about 25 g were used in this study. After anesthetization, full-thickness rectangular wound of 1 cm × 1 cm were prepared on the backs of rats parallel with the vertebral column. The COS-loaded PVA sponge was applied on the wounds of each rat. The same wound was also prepared to treat with the cotton gauze as a control. At the fourth postoperative day, macroscopic photographs were obtained from the wounds. The wound area was measured using a slide caliper at the eighth postoperative days after initial wounds, and the wound was taken for examining the epithelialization and granulation through the histological observation.

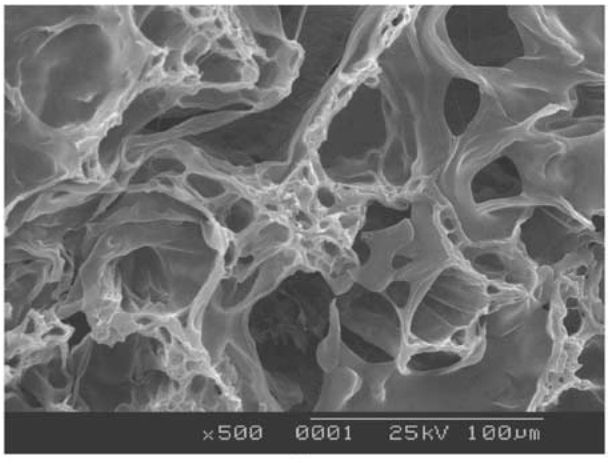
3. Results and discussion

3.1. Morphology of PVA sponges

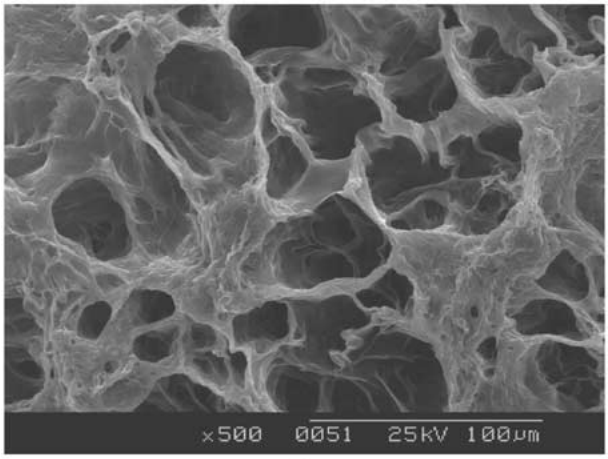
After lyophilization of PVA/COS solution, elastic and soft sponges were obtained. Fig. 1 shows representative SEM images for the surface of COS-loaded PVA sponges (PVA/COS = 50/50, w/w). The sponges seemed to have the inter-connected network structure. As the concentration of COS-loaded PVA sponge increased from 1% to 3%, the pore size of sponge became smaller (from 65 μm to 28 μm) as shown in Fig. 2. Average pore size was calculated by measuring the size of 50 pores by Image Measuring & Processing Software (Scope Eye). For the PVA sponge prepared at the same concentration, pore size also decreased, as the content of COS to PVA increased from 100/0 to 50/50. In view of cross section, the other sponges showed almost similar morphology. As expected, the morphology of COS-loaded PVA sponges was strongly dependent on the mixing ratio and PVA concentration.

3.2. *In vitro* COS release behavior

Fig. 3 shows the COS release behaviors of PVA sponges loaded with different amount of COS. The COS release from PVA sponge was relatively fast and showed considerable release within 10 h, irrespective of the



(a)



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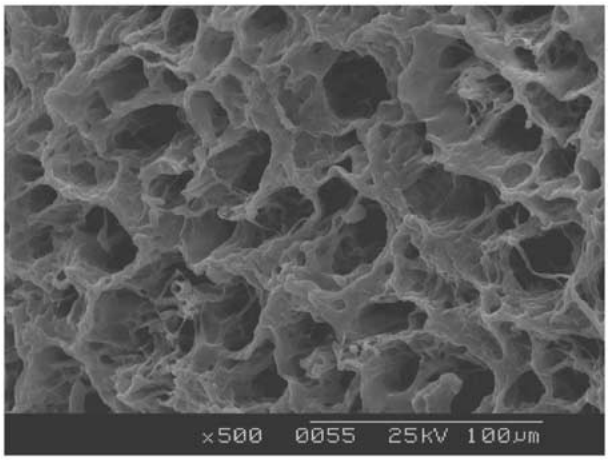


(c)

Figure 1 SEM images for the surface of COS-loaded PVA sponges (PVA/COS = 50/50, w/w): (a) conc. 1%, (b) conc. 2%, (c) conc. 3%.

COS content in the sponge. In addition, PVA concentration slightly affects the release rate of COS. From this result, the interaction between the COS and the PVA matrix seemed to be relatively weak. However, the release rate of COS from PVA sponge *in vivo* was expected to be much slower than *in vitro*, because the environment at the wound was less moistened than in the PBS solution. For the antimicrobial and wound-healing test, we chose COS-loaded PVA sponges prepared at the concentration of 1% as suitable sponge samples. All the subsequence results are for PVA samples prepared in this concentration.

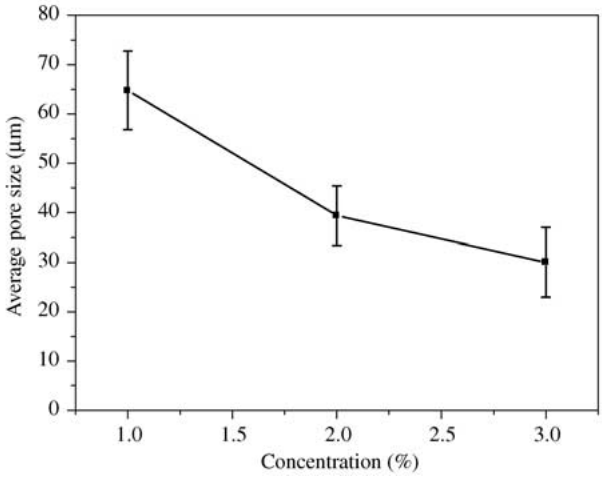


Figure 2 Average pore size versus concentration of COS-loaded PVA sponge.

3.3. Antimicrobial activity of PVA/COS sponge

Antimicrobial activity of the COS-loaded PVA sponge is considered to be one of the most important properties linked directly to the wound dressing. Two mechanisms were proposed for the antimicrobial activity by chitosan and its derivatives. One mechanism is that the polycationic nature of chitosan interferes with bacterial metabolism by stacking at the cell surface [12]. The other is blocking of description to RNA from DNA by adsorption of penetrated chitosan to DNA molecules [8]. In this mechanism molecular weight of chitosan must be less than around some critical value (≈ 5000) to permeate into the cell.

The antimicrobial activity of the PVA sponges with different COS content was examined against a representative gram-positive bacterium, *S. aureus*, which causes pimples on the human skin. The activity data were evaluated in terms of the suppression percentage of the colony formation. As shown in Table I, the COS-loaded sponges showed a considerable bactericidal activity irrespective of the COS content, whereas that without COS was somewhat less active. Therefore, the antibacterial actions exerted by the COS-loaded PVA sponge appears to be an important additional feature of wound dressing because it could contribute to the

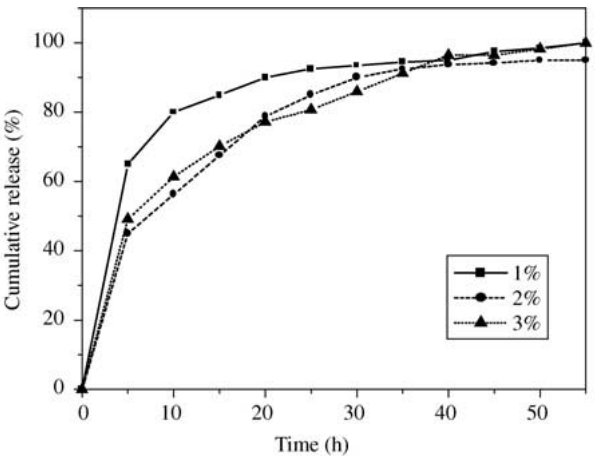


Figure 3 COS release behavior from COS-loaded PVA sponges (PVA/COS = 50/50, w/w).

TABLE I Antimicrobial activity of PVA sponges with or without COS against *S. aureus*

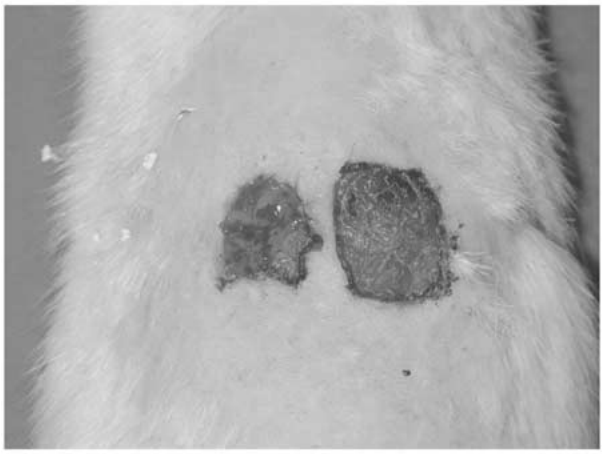
	PVA sponge	PVA/COS sponge (90/10)	PVA/COS sponge (70/30)	PVA/COS sponge (50/50)
Suppression of the growth (%)	34 ± 3	$> 99 \pm 0$	$> 99 \pm 0$	$> 99 \pm 0$

prevention of secondary infections in wounds by *S. aureus*, resulting in limited scar formation.

3.4. Wound healing

In open wound-healing test, twin full-thickness rectangular wounds were made on the back of each rat. The

(a)



(b)

(c)

Figure 4 Macroscopic observation of wound at fourth day after initial wounding: (a) PVA/COS (90/10), (b) PVA/COS (70/30), (c) PVA/COS (50/50).

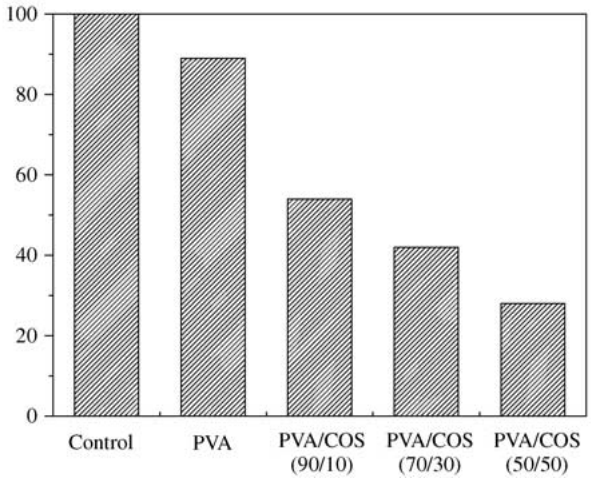


Figure 5 Wound areas of PVA sponges with different COS content at eighth day after initial wounding.

COS-loaded PVA sponge appeared to stop bleeding at the wound site and thus acted as a hemostatic agent. Fig. 4(a)–(c) shows the wounds observed macroscopically at fourth day after initial wounding. In comparison with the control wound treated with cotton gauze, wound closure the COS-loaded PVA sponges was faster. Significant differences in wound closure between the COS-loaded PVA sponges with different COS content were seen on the eighth day, although not on fourth day. Fig. 5 shows wound areas of the PVA sponges with different COS content. As the COS content in sponge increased from 0% to 50%, the average wound area decreased gradually from 90.8% to 27.7%, indicating that the COS accelerated wound-healing.

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