Evaluation of Chitin and Chitosan on Open Wound Healing in Dogs

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ABSTRACT. Analysis on the accelerating effects of open wound healing by chitin and chitosan were carried out in dogs. Two, square, full-thickness wounds of skin (2 × 2 cm²) were created on the each dog’s both sides of dorsal midline at 0, 14, 21, and 24 days. In one dog, one wound (left side) was treated with chitin (chitin group) and the other wound (right side) was not treated (control group). In another dog, one wound (left side) was treated with chitosan (chitosan group) and the other wound (right side) was not treated (control group). At 28 days after initial wounding, each wound site including surrounding tissue was taken for macroscopic and histological observations. Reepithelialization tended to be greater in chitin and chitosan groups than in the control group. However, when the scores of reepithelialization and granulation tissue were evaluated statistically, there was no significant differences in three groups during experimental period. Number of inflammatory cells was greater statistically in level in the control group than those in chitin and chitosan groups at 28 days after wounding. Many rete ridges were observed in the control group but very few in the another groups. — KEY WORDS: canine, chitin and chitosan, open wound healing, rete ridge.


In general, open wound heals by inflammation and the cellular responses, leading to granulation tissue formation, reepithelialization, and wound contraction [1, 3]. A variety of wound medications has been used and investigated in the treatment of open wound. Many of these medications may allow normal wound healing but none actually stimulate or accelerate healing. A series of our studies indicated that chitin and chitosan, polymers of N-acetyl-D-glucosamine and D-glucosamine, accelerated wound healing in many clinical cases [7–10, 13, 15], and that chitin induced granulation tissue with angiogenesis and inflammatory cells [14]. Our in vitro study indicated that chitin and chitosan induced migration of bovine polymorphonuclear cells [17]. Some studies indicated that chitin was efficient on human cutaneous wounds [12]. Chemically modified chitosan including N-carboxybutyl chitosan [2] and acetic acid chitosan [6] were efficient on cutaneous wound. However, little is known about detail effects of chitin and chitosan on open wound healing.

The purpose of this study was to evaluate effects of chitin and chitosan on open wound healing in dogs.

MATERIALS AND METHODS

Animals: Eight adult mongrel dogs, 3 females and 5 males, weighing 8–10 kg were used in this study. Chitin and chitosan: Chitin (less than 30% deacetylated) and chitosan (more than 80% deacetylated) granules, 3 μm on mean size (range from 0.5 to 10 μm), were supplied by Sunfive Co., Ltd. (Tottori, Japan). These materials were sterilized by ethylene oxide gas before use.

Creation of open wound: The animals were anesthetized with sodium pentobarbital (25 mg/kg i.v.) after atropine sulfate (0.05 mg/kg s.c.) and buprenorphin-HCl (5 μg/kg s.c.) premedication. Each dog received two, square, full-thickness wounds of skin (2 × 2 cm²) on the each dog’s both sides of dorsal midline at 0, 14, 21, and 24 days (Fig. 1). In one dog, one wound (left side) was treated with chitin (chitin group) and the other wound (right side) was not treated (control group). In another dog, one wound (left side) was treated with chitosan (chitosan group) and the other wound (right side) was not treated (control group). All wounds were covered with a non-adherent occlusive bandage. Bandage was change every 2 days, and chitin and chitosan were reapplied every 2 days.

Macroscopic and histological observations: At 28 days after initial wounding, each wound was taken for macroscopic and histological observations. These tissues were then fixed in 10% phosphate-buffered formalin.

Fig. 1. Two, square, full-thickness wounds (2 × 2 cm²) on both sides of the dorsal midline of each dog were created at 0, 14, 21 and 24 days. In one dog, one wound (left side) was treated with chitin (chitin group) and the other wound (right side) was not treated (control group). In another dog, one wound (left side) was treated with chitosan (chitosan group) and the other wound (right side) was not treated (control group).
Samples were then dehydrated, embedded in paraffin and sectioned into 3- to 4-μm thick specimens before staining with hematoxylin-eosin and Masson's trichrome reagents. The wounds created at 24, 21, 14, 7 and 0 days were named post-wounding day (PWD) 4, 7, 14 and 28, respectively. Macroscopic and histological evaluations were performed by modified method of Tsuboi and Rifkin [16]. Degree of reepithelialization and granulation tissue formation were rated on a scale of 0 to 3 or 4 as follows: degree of reepithelialization; 0=not found, 1=less than 30%, 2=more than 31% and less than 60%, 3=more than 61% and less than 99%, and 4=complete, granulation tissue formation; 0=not found, 1=thin, 2=moderate, and 3=full thickness. Numbers of inflammatory cells, fibroblasts, and neovascularization were also rated on a scale of 0 to 3 as follows: 0=normal, 1=slight, 2=moderate, and 3=severe.

Statistical analysis: All data were analyzed by Mann-Whitney's U test. Results were expressed as a mean ± s.e.

RESULTS

Macroscopic observations of the open wound: Macroscopic findings of open wound are summarized in Fig. 2. In all groups, granulation tissues at the wound were observed on PWD 4, subsequently the wounds were filled with granulation tissues on PWD 7. On PWD 28, complete reepithelialization had developed in all cases in the chitin group and 3 out of 4 cases in the chitosan group, respectively, but did not develop in 3 out of 8 cases in the control group. When the scores of reepithelialization and granulation tissue were evaluated statistically, there was no significant differences in three groups. A considerable amount of exudate appeared on the wound surface and the wound margin became thickened in the chitin and chitosan groups on PWDs 4 and 7. In the control groups, a slight amount of exudate appeared on the surface and some wound surfaces were dry at the same time. Wound contraction tended to be greater in the chitin and chitosan groups than in the control groups.

Histological observations of the open wound: Histological findings of the open wound are summarized in Fig. 3. Fibroblasts and newly formed capillaries proliferated more prominently at the bottom of wounds in the chitin and chitosan groups than in the control group on PWD 4, but no significant difference. In all groups, the degrees of inflammatory cells infiltration and neovascularization and the number of fibroblasts were peak on PWD 7 and 14, respectively. On PWD 28, the scores of inflammatory cells in the chitin and chitosan groups were significantly decreased in comparison to that in the control group (p<0.05). At this time, collagen fibers synthesized were

![Image](image_url)

**Fig. 2.** Macroscopic findings of open wound. Scoring of each parameter was graded by the method described in this paper.

![Image](image_url)

**Fig. 3.** Histological findings of open wound. *Value is different from that of control, p<0.05. Scoring of each parameter was graded by the method described in this paper.
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Fig. 4. Post wounding day 28. Thickness of epidermis was almost constant in the chitin and chitosan groups. × 350, hematoxylin-eosin stain (HE).

Fig. 5. Post wounding day 28. Many rete ridges were observed in the control groups. × 350, HE.

Fig. 6. Post wounding day 28. Moderate infiltration of inflammatory cells was observed in the control groups. × 350, HE.

finer in the chitin and chitosan groups than in the control group, and thickness of epidermis was almost constant in the chitin and chitosan groups (Fig. 4), while many rete ridges were observed in the control group (Fig. 5). At this time, slight or moderate infiltration of inflammatory cells was observed locally in all cases in the control group (Fig. 6), while slight infiltration of them was observed locally in each one out of 4 cases in the chitin and chitosan groups.

DISCUSSION

From the present study, chitin and chitosan found to reconstitute normally epidermal structure. In the control group, many rete ridges were observed but not in the chitin and chitosan groups, so that epidermis was more smooth in the chitin and chitosan groups than that in the control group. Rete ridges were found in various dermatitis [19] but not in normal haired skin of domestic animals, except for pig [19]. So, rete ridges would be formed due to degree of stimuli. The fact that numbers of inflammatory cells were significantly less in the chitin and chitosan groups than in the control group on PWD 28 (Fig. 3) supports that chitin and chitosan would give less stimuli and regenerate normal skin in open wounds. Ohshima et al. [12] reported that excessive scar formation did not develop in the presence of chitin. Our previous studies also indicated the similar evidence.

Macroscopically, chitin and chitosan had a tendency to enhance reepithelialization but no statistical difference in three groups during experimental period. Open wounds heal by inflammation and cellular responses, leading to the granulation tissue, the proliferation and migration of epithelial cells, and wound contraction [1, 3]. A series of our previous studies indicated enhancement of inflammatory cells activities and induction of granulation tissues accompanying with angiogenesis in the presence of chitin and chitosan [8-10, 13-15]. Ohshima et al. [12] reported that chitin dressing showed excellent results when it was applied for dressing of donors sites, skin graft areas, burns, ulcer, and so on. The macroscopic findings, however, could not support clearly those data.

The present study indicated that chitin and chitosan induced exudate as well as our previous studies [13]. Recent studies indicated that wound fluid in acute phase play a role as accelerator for wound healing [4]. Winsteadley [18] showed that epithelial cells migrate into the exudate rather than down the dermal wall in dogs. The present results, however, did not showed clearly relationship the exudate induced by chitin and chitosan and wound healing including...
reepithelialization. In the future, further investigation is necessary in this respect.

Microscopically, collagen fibers synthesized were thinner in the chitin and chitosan groups than those in the control group. Kishimoto and Tamaoki [5] reported that fine collagen fibers were induced in the presence of chitin. The present results is coincide with the previous data.

In wound healing process, there is no report in comparison to chitin and chitosan. Chitosan was more efficient in dirty wound including abscess, bite wound, and traffic accident than chitin [9]. In the present study, however, there was no statistical difference macroscopically and histologically between chitin and chitosan groups (Figs. 2, 3).

REFERENCES


