Application of Polymeric N-Acetyl-D-Glucosamine (Chitin) to Veterinary Practice

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ABSTRACT. The sponge-, cotton-, and flake-type remedies made of chitin (chitin-sponge, chitin-cotton, and chitin-flake, respectively), and non-woven fabric of polyester (NWF) composed with chitin (chitin-NWF) were applied to various types of trauma, abscess, surgical tissue defect and herniorrhaphy in 147 clinical cases including 72 dogs, 38 cows, 33 cats, 2 rabbits, one monkey and one horse. Chitin-sponge was applied in 30 cases as filling agent of surgical tissue defect, and in 25 cases of trauma, 31 cases of abscess as wound dressing or tissue defect filling agent. In 77 out of 86 cases (89.5%), good healing developed. When chitin-sponge was buried in surgical tissue defects due to oncotomy in 20 cases, recurrence of the tumor developed in one case on one month post-operatively, but was not recognized for 3-24 months in 19 cases. Chitin-NWF was applied in 2 cases of trauma and 12 cases of abscess as wound dressing or tissue defect filling agent. 6 cases as filling agent of surgical tissue defect, and 12 cases of umbilical hernia as prosthesis of suture site of hernia ring. In 28 out of 32 cases (87.5%), good healing developed. Chitin-cotton was applied in 8 cases of trauma and 12 cases of abscess as wound dressing or tissue defect filling agent. In 18 out of 20 cases (90.0%), good healing developed. Chitin-flake was applied in 9 cases of trauma as wound dressing or tissue defect filling agent. In 8 out of 9 cases (88.9%), good healing developed. In all cases, no side effects were observed.—Key words: biomaterial, chitin, filling agent, wound dressing agent, wound healing.


Various types of wound remedies have been developed for the purpose of enhancing wound healing. These agents are classified mainly into those made of synthetic products [7, 8, 21, 24] and of natural products [2, 3]. Recently, much attention has been paid to various biological wound dressing agents made of natural materials because of their high biocompatibility. However, these agents were not able to completely fulfill all functions as wound dressing agents.

A biological filling agent for the promotion of wound healing in tissue defect is required not only in the treatment of abscesses, but also in the treatment of surgical tissue defects caused by oncotomy or reducing hernia. Conventional filling agents, such as artificial mammmae, artificial eyes, artificial noses and so on are utilized only to maintain the shape in the face of a particular soft tissue defect in human medicine, and there is little biological filling agent buried in a wound cavity about the promotion of wound healing.

For these reasons, further investigations have been carried out on the material, nature, shape and fitness of the wound dressing and filling agents. Beschit W (Unitiika Co., Ltd., Kyoto, Japan), non-woven fabric of polymeric N-acetyl-D-glucosamine (chitin), is one of the artificial types of skin developed for these purposes [9]. Chitin is distributed widely in nature as the skeletal material of crustacean, insect, fungus, and the cell walls of bacteria and is biodegraded by some enzymes [1, 22] as same as cellulose which has similar structure to chitin. Chemically modified chitins including partially deacetylated and carboxymethylated chitins were found to have potent immunological activities [6, 13-18]. Recently, the studies we conducted have indicated that unmodified chitin has medically utilisable biological activities including the acceleration of granulating tissue formation and production of some biomedical substances at a level similar to that of chemically modified chitin [11, 12, 19].

In the present study, the effects of biological filling and wound dressing agents made of unmodified chitin were evaluated for application to various traumas, abscess, prosthesis of hernia, and surgical tissue defect.

MATERIALS AND METHODS

Preparation of biological filling and wound dressing agents: A composite sheet was made of chitin obtained from the pen of Neon flying squid (Ommastrephes bartramii) (Nippon Suisan Co., Ltd., Tokyo, Japan) and a non-woven fabric of polyethylene terephthalate (NWF) (SN-1015H; Teijin Co., Ltd., Tokyo, Japan). Chitin (1.5 g) was dispersed in 100 ml of water, and 1 ml of the aliquot was added to 500 ml of methanol. The suspension was filtered through NWF (15-cm diameter) over a Nütsche funnel. After removing the water with some pieces of filter paper, the chitin/NWF complex (chitin-NWF) was air-dried at room temperature.

Sponge-type chitin was made of chitin obtained from the pen of Neon flying squid as follows. Using a mixer, a 1.5% (W/V) dispersion of chitin in water was prepared. Then, 5.5 ml of the dispersion prepared was put into a petri dish (40 mm in diameter and 5 mm in height) made of polystyrene, and frozen at -20°C, followed by freeze-drying for a whole day and night, resulting in a sponge-type chitin (chitin-sponge).
Cotton-type chitin was produced by pulverizing chitin obtained from the pen of Neon flying squid with use of an ACM Pulverizer 10 manufactured by Hosokawa Micron Co., Ltd. (Osaka, Japan). The pulverization was conducted using a bar-shaped hammer and a grooved liner for 25 min at a rotational frequency of 6,800 rpm with a rated current of 24 amperes. The obtained cotton-type chitin (chitin-cotton) had an apparent specific gravity of 0.05 to 0.13 g/cm³ and was in the form of fibers 0.1 to 0.8 mm in length and 10 to 120 μm in width.

Flake-type chitin (chitin-flake) was purchased from Nippon Suisan Co., Ltd. (Tokyo, Japan).

These agents were sterilized with ethylene oxide gas before use.

Application of chitin agents to clinical cases: Four types of chitin agents were applied in 147 clinical cases as biological filling and wound dressing agents. The animals included 72 dogs, 38 cows, 33 cats, 2 rabbits, one monkey and one horse.

In the cases of surgical tissue defect due to oncotomy, herniorrhaphy or another operations, the chitin-sponge or chitin-NWF was used as filling agent. In the case of an intrapelvic tissue defect due to the reduction of perineal hernia, the chitin-NWF was wound up into a roll form so as to fit with the wound cavity along the rectum.

In the treatment of umbilical hernia, the chitin-NWF was buried in the subcutaneous portion as it had a sheet form, as a prosthesis for the stitches of the hernia ring, and the peripheral portion of the sheet was fixed by an interrupted suture.

In the treatment of abscess and various types of trauma including bite wounds, skin erosion or sores, and skin problems arising as a result of traffic accidents, the chitin-sponge, chitin-NWF, chitin-cotton or chitin-flake was used as tissue defect filling or wound dressing agent. At initial therapy, a suitable chitin agent was buried into wound cavity or was covered on wound surface following lavage and disinfection but not debridement, and thereafter was exchanged several times until the trauma and abscess healed. All animals were treated under general or local anesthesia, whenever necessary. Antibiotic was administered systemically in some animals which had systemic symptoms such as fever, anorexia and so on, but was not done locally in all cases.

The systemic and/or topical responses after treatment were observed macroscopically until they were cured or the local symptoms disappeared.

It was judged that the chitin agent was efficient, when the wound healed without side effects or recurrence of the tumor was not recognized.

RESULTS

The effects of chitin agents on various diseases are summarized in Table 1.

Chitin-sponge was applied in 30 cases including 20 cases of tumor, 4 cases of hernia, 2 cases of hematoma, 2 cases of patellar luxation, one case of traumatic teeth injury and one case of castrating as filling agent of surgical tissue defect (Fig. 1), and in 25 cases of trauma, 31 cases of abscess as wound dressing or tissue defect filling agent. In 77 out of 86 cases (89.5%), good healing developed. When chitin-sponge was buried in surgical tissue defect due to oncotomy in 20 cases, recurrence of the tumor was not recognized for 3–24 months in 19 cases. In one case, recurrence of the tumor developed after one month post-operatively. The tumor was placed subcutaneously on the buried chitin-sponge and so was easily removed.

Chitin-NWF was applied in 2 cases of trauma as tissue defect filling agent, 12 cases of abscess as wound dressing agent, 6 cases including one case of tumor, 4 cases of hernia and one case of hematoma as filling agent of surgical tissue defect, and 12 cases of umbilical hernia as prosthesis of suture site of hernia ring. In 28 out of 32 cases (87.5%), good healing developed. Recurrence of the hernia did not develop in all cases. However, in both two cases of trauma and one case of hernia developed after removal of the chitin-NWF.

Chitin-cotton was applied in 8 cases of trauma and 12...
cases of abscess as tissue defect filling or wound dressing agent (Fig. 2). In 18 out of 20 cases (90.0%), good healing developed.

Chitin-flake was applied in 9 cases of trauma as tissue defect filling or wound dressing agent. In 8 out of 9 cases (88.9%), good healing developed.

In these cases of injuries including traumas and abscesses, formation of healthy granulating tissue was observed within one week after treatment. Skin defects subsequently reepithelialized without scar formation and any functional disturbances. In 6 out of the 44 cases of trauma, and in 7 out of the 55 cases of abscesses, granulating tissue did not develop. In these cases, general conditions were serious and contamination of the wounds was severe.

The effects of chitin agents on the various animals are summarized in Table 2. In dogs, cats, and cows, the wound healing effects of these chitin agents were almost the same.

**DISCUSSION**

Chitin was found to accelerate wound healing in various types of trauma and abscesses, in which healthy granulating tissue developed after treatment. It is well known that healthy granulating tissue develops only in the absence of foreign bodies such as bacteria, debris, and so forth [4]. Although chemically modified chitin such as 30% and 70%-deacetylated chitin (DAC-30 and DAC-70, respectively) effectively enhanced non-specific host resistance to *E. coli* infection, unmodified chitin did not enhance such resistance and so did not show the same antibacterial function [13]. On the other hand, we reported that unmodified chitin accelerated the granulating tissue formation [11, 12, 19] and the production of some biomedical substances [19]. The present results indicate that chitin agents may attain excellent advantages from the fact that various cells showing a biophylaxis function are allowed to migrate for the purpose of fighting the bacteria and treating the necrosing tissue in the wound.

The present agents did not develop almost scar tissue that causes functional disturbances. Scar formation depends on both continued collagen synthesis and collagen catabolism [4]. The degradation of wound collagen is initiated by a variety of collagenase enzymes from granulocytes [20], macrophages [26], epidermal cells, and fibroblasts [5]. Kishimoto and Tamaki reported that renewed collagen fibers in the wound were fine in the presence of chitin in guinea pigs [9]. Moreover, Yano et al. reported that collagen synthesis was inhibited in the presence of chitin in rats [27]. The present results generally support their data. It may be that the release of collagenase enzyme from inflammatory cells including granulocytes, macrophages, epidermal cells, and fibroblasts is enhanced in the presence of chitin.

Chitin had similar effects of the wound healing for various animals mentioned above. In addition, when these agents were applied to various types of trauma and abscesses as filling or wound dressing agent, in spite of the different shapes of chitin agents, similar effects such as the healthy granulating tissue formation were induced. In preliminary experiments, the paper or film type remedy made of chitin did not accelerate wound healing because they did not maintain contact with the wounds. However, the present materials kept in contact with the wounds. These results suggest that the shape of the chitin agent affects the healing of wounds, and that keeping chitin in contact with the wounds is important.

The chitin-sponge and chitin-NNWF were found to be
efficient as filling agent for surgical tissue defect. Conventional filling agents are used only in plastic therapy. As substitute materials for maintaining shape, synthetic products such as silicone, vinyl chloride, and styrene foam are usually used. These materials are only buried in an organism to physically regulate shape and they do not promote wound healing. In many surgical tissue defect, it is desirable for the material buried to be biodegradable and to be replaced by native organisms. We could not observe the fate of the filling agents at the site of a surgical tissue defect. However, it was assured that the chitin-sponge was almost completely biodegraded within two weeks and did not develop the scar tissue when it was implanted subcutaneously in dogs [unpublished data] and chitin-NWF was organized accompanying angiogenesis [11]. In the case of perineal hernia, it was guessed that recurrence of the hernia did not develop because of organization of the chitin-NWF.

It was suggested that chitin suppressed recurrence of tumor. Hexa-N-acetylchitohexaose and chitohexaose, consisting soley of N-acetyl-D-glucosamine and D-glucosamine, respectively [23], and DAC-30 and DAC-70 [13] inhibited growth of Meth-A in mice, but unmodified chitin did not [13]. In the future, long-term observation and further investigation are necessary in respect to effects of chitin for recurrence of the tumor.

Chitin-NWF was also found to be beneficial as prosthesis. Synthetic materials of high tensile strength have been used for the repair of abdominal wall defects in large animals [8, 10, 24, 25]. Polypropylene meshes have been
described most frequently for it [8, 24]. However, operative method using these materials is complicated. Operative method was more simple using chitin-NWF than another materials.

In conclusion, it is assumed that these chitin biological filling and wound dressing agents are sufficiently effective for veterinary practice.

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