

## Chitosan Derivative-based Hemostat: Irritation, Hypersensitivity and Absorbability Study

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### Abstract

The objective of this study was to evaluate the safety of a carboxymethylchitosan-based hemostatic prototype for internal use, in terms of irritation, hypersensitivity and absorbability. Skin irritation and delayed-type hypersensitivity tests of the prototype in humans (n=30) were assessed by a patch test method. The human skin sites were graded and photographed after patch removal at each time point using a 4-point (0-3+) grading scale of increasing irritation related to ISO 10993-10:2002(E) and a 6-point grading scale of increasing allergy related to International Contact Dermatitis Research Group (ICDRG). The results demonstrated that the chitosan derivative-based hemostat did not cause skin irritation and delayed-type hypersensitivity on the tested humans' skin. The absorbability of the hemostatic prototype was evaluated in the surgical treatment of penile paraffinoma in ten patients, which was conducted at Anghong Hospital, using a scrotal flap method. This method normally involved two surgical stages. In the first stage, two prototype specimens (2.5 cm x 2.5 cm x 0.20 cm) were implanted inside a patient's treated area until the second operation was performed, normally in three months. The clinical results revealed that, after the whole treatment, no remaining specimens were physically observed. Furthermore, the histological results of the tissues where the specimens had been implanted showed no sample residues, indicating that the chitosan derivative-based hemostat was completely absorbed within three months.

**Key words :** Clinical, Chitosan Derivative-based Hemostat, Irritation, Hypersensitivity, Absorbability

### Introduction

Carboxymethylchitosan, a water-soluble derivative of chitosan, was proved to be non-cytotoxic, biocompatible, and biodegradable.<sup>(1,2)</sup> It has found various uses in biomedical applications, particularly as wound healing and hemostatic materials.<sup>(3,4,5)</sup> In our previous study, both *in vitro* and *in vivo* hemostatic abilities of a carboxymethylchitosan-based hemostatic prototype were assessed with respect to that of a commercial material, SPONGOSTAN<sup>®</sup> Standard. The results revealed that the prototype could significantly shorten the whole blood clotting time (WHBCT),  $p < 0.05$ , with a hemostatic efficacy comparative to that of SPONGOSTAN<sup>®</sup> Standard.<sup>(3)</sup> In the animal trial, the prototype, however, stopped the bleeding from the transected rat tails more effectively than SPONGOSTAN<sup>®</sup> Standard.<sup>(6)</sup> Furthermore, the wound healing efficacy and resorbability of the prototype were also evaluated in rats. Nevertheless,

few studies on biological safety of this chitosan derivative in both *in vitro* and *in vivo* models were reported.<sup>(7,8)</sup> Moreover, the degradation and absorbability of carboxymethylchitosan-based products in the human body have rarely been reported, especially in hemostatic application.

The objective of this study was, therefore, to clinically evaluate the safety of the carboxymethylchitosan-based hemostatic prototype for internal use, in terms of skin irritation and hypersensitivity and absorbability. Skin irritation and delayed-type hypersensitivity was assessed by using a patch test, whereas the absorbability of the hemostatic prototype was examined in the surgical treatment of penile paraffinoma in ten patients who had sclerosing lipogranuloma. The degradability and absorbability of the implanted prototype were investigated by visual observation and histopathological examination of the surrounding tissues.

## Materials and Experimental Procedures

### *Materials and Methods*

The studies on the skin irritation and delayed-type hypersensitivity and absorbability of the hemostatic prototype were approved by the Independent Ethics Committees of Anghong Hospital, No.AEC.001/53 and No.AT.0027.2025/001, respectively. The trials were conducted during April 2010-February 2012. The consent forms were signed by the patients upon their enrollment in the trials.

### *Preparation of Carboxymethylchitosan-based Hemostatic Prototype*

Briefly, highly viscous aqueous solution of carboxymethylchitosan-based hemostat was poured into molds with a given dimension and subsequently lyophilized to produce sponge-like pads. The water-soluble sponges were then individually immersed into gently stirred 10 wt% aqueous calcium chloride solution for 30 min to produce the water-insoluble pads. Afterwards, the pads were removed and successively washed with deionized (DI) water. The resulting pads were ultimately freeze-dried to yield chitosan derivative-based hemostatic prototype which was sterilized by ethylene oxide gas prior to use.

### *Human Skin Irritation and Delayed-type Hypersensitivity study*

Carboxymethylchitosan-based hemostatic prototype (2.5 cm x 2.5 cm x 0.15 cm) was wetted with DI water (0.4 ml). Two controls were employed: gauze (2.5 cm x 2.5 cm x 0.3 cm) wetted with DI water (0.4 ml), as a negative control, and gauze wetted with 15%w/v sodium dodecyl sulfate (SDS) (0.4 ml), as a positive control. The human skin allergic and irritant patch test was conducted in 30 healthy volunteers (17 females and 13 males, aged 24–68 years). All three test samples were applied to each volunteer's upper outer-arm with the duration of exposure to the test samples increasing progressively from 1, 2, 3, 4, 24, 48 to 72 hours. The skin reactions (erythema and oedema) were graded after patch removal at each time point using a 4-point (0-3+) grading scale of increasing irritation related to ISO 10993-10: 2002(E)<sup>(9-10)</sup> and a 6-point grading scale of increasing allergy related to International Contact Dermatitis Research Group (ICDRG). The acquired data were

statistically analyzed using a paired t-test, and  $p < 0.001$  was significantly considered.

### *Degradability and Absorbability Study*

The degradability and absorbability of the carboxymethylchitosan-based hemostatic prototype was examined in the surgical treatment of penile paraffinoma in ten (male) patients aged 18-60 years using a scrotal flap method. They were referred with different acute symptoms after injection of foreign substances, i.e., Vaseline or olive oil, into penises for the purpose of augmentation. Generally, at surgical reconstruction, complete excision of the involved penile skin was performed, followed by skin substitution from the existing and saved coat of the penis. Two pieces of the hemostatic prototype (2.5 cm x 2.5 cm x 0.20 cm) were implanted at the right and left scrotum flaps of the penis and left inside the body until the second operation which was in about three months. The implant surrounding tissues were subsequently sampled and sent out to HI-TECH LAB Co. Ltd (Sala Daeng, Bangkok, Thailand) for histopathological examination and interpretation.

## Results and Discussions

### *Skin Irritation and Delayed-type Hypersensitivity of the Hemostatic Prototype*

The clinical results of human skin irritation and delayed-type hypersensitivity of the carboxymethylchitosan-based hemostatic prototype assessed by using a 4-point grading scale of increasing irritation and a 6-point grading scale of increasing allergy are revealed in Table 1. It was found that 29 of 30 volunteers had irritant and allergic skin reactions to the gauze wetted with 15%SDS (positive control) at 72 h, whereas there was no irritant and allergic skin reactions to the gauze wetted with DI water (negative control) and hemostatic prototype in all tested volunteers at 1-72 hours. In addition, the percentages of cumulative skin irritation reaction to 15%SDS (positive control) at 4, 24, 48 and 72 hours were 10.00%, 83.33%, 90.00% and 96.67%, respectively.

**Table 1.** The percentage of cumulative irritation of various materials at different exposure times.

| Exposure time (h) | Number of irritated subjects (%Cumulative skin irritation) |  |   |
|-------------------|--|--|---|
|                   | Carboxymethylchitosan-based hemostatic prototype           | Positive control (gauze wetted with 15% SDS) | Negative control (gauze wetted with DI water) |
| 1                 | 0(0%)  | 0(0%)  | 0(0%)   |
| 2                 | 0(0%)  | 0(0%)  | 0(0%)   |
| 3                 | 0(0%)  | 0(0%)  | 0(0%)   |
| 4                 | 0(0%)  | 3(10.00%)                                    | 0(0%)   |
| 24                | 0(0%)  | 25(83.33%)                                   | 0(0%)   |
| 48                | 0(0%)  | 27(90.00%)                                   | 0(0%)   |
| 72                | 0(0%)  | 29(96.67%)                                   | 0(0%)   |

As shown in Table 2, the mean irritant grading scales of the carboxymethylchitosan-based hemostatic prototype were statistically significantly different from those of the positive control group ( $p < 0.001$ ) at 24, 48 and 72 hours. These results designated that carboxymethylchitosan-based hemostat was safe for clinical use in humans as it did not induce any skin irritation and delayed-type hypersensitivity.

**Table 2.** Statistical comparison between carboxymethylchitosan-based hemostat and positive control.

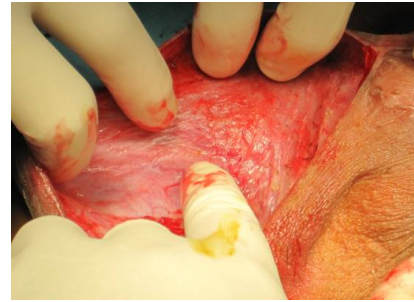
| Race comparison                                     | <i>p-value</i> |
|---|----------------|
| hemostatic prototype versus positive control (4 h)  | 0.083          |
| hemostatic prototype versus positive control (24 h) | 0.000*         |
| hemostatic prototype versus positive control (48 h) | 0.000*         |
| hemostatic prototype versus positive control (72 h) | 0.000*         |

\* $p < 0.001$

**Absorbability of the Hemostatic Prototype**

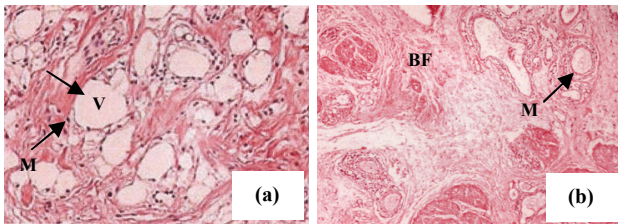
As vividly revealed in Figure 1, after the chitosan derivative-based hemostatic prototype had been implanted at the right and left scrotum flaps of the patient’s penis for three months, both pieces

of the prototype specimens were absolutely degraded. No trace of specimens was visually found.



**Figure1.** Photograph of the penile sites where two prototype specimens had been implanted for three months.

The results on histopathological analysis of tissue biopsies before and after implantation of the prototype are shown in Figure 2. Figure 2(a) illustrates the biopsied penile tissue before implantation. It was found that there was intradermal infiltration of mononuclear inflammatory cells and numerous vacuoles (coded as V) of various sizes in the dermal tissue. Numerous epithelioid histiocytes were extensively observed. Furthermore, the spaces were surrounded by multinucleated giant cells (coded as M), similar to the diagnosis of testicular sclerosing lipogranuloma reported previously.<sup>(11)</sup> The histopathological photomicrographs of the biopsied tissue after implantation are shown in Figure 2(b); benign fibrovascular (coded as BF) and adipose tissue existed. There were scattered small blood vessels with mild mononuclear leukocytic infiltrates and focal aggregates of multinucleated gaint cells. Moreover, the inflammatory cells with a lipoid granuloma that was the same type of cells found in the biopsied tissue before implantation was also present. This was likely that the penile paraffinomas were not completely excised during the first surgical stage of the scrotal flap method. However, no foreign body material and inflammation of the tissues were observed. These results suggested that carboxymethylchitosan-based hemostatic prototype did not induce foreign bodies and inflammation of the tissues upon degradation and was ultimately absorbable. The histopathological photomicrographs of the biopsied tissues after implantation from all ten patients were virtually identical; no foreign body material was found.



**Figure 2.** Histopathological photomicrographs of penile tissues: (a) before and (b) after implantation of tested specimens, original magnification  $\times 200$  (V = vacuole, M = multinucleated giant cells, and BF = benign fibrovascular).

## Conclusions

The safety of carboxymethylchitosan-based hemostatic prototype for internal use was assessed. The results of this study clearly demonstrated that the prototype did not cause skin irritation reactions to all thirty volunteers throughout the 72-h patch test. The mean irritant grading scales of hemostatic prototype were statistically significantly different from those of the positive control group ( $p < 0.001$ ) at 24, 48 and 72 h. In addition, the implantation results revealed that the hemostatic prototype was completely decomposed within 3 months in the penises of all ten patients who had Vaseline or olive oil induced sclerosing lipogranuloma. No foreign body materials and inflammation of the tissues were noticed.

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