

# Gelatin Sponge Sheet Combined with Gelatin Glue as New Topical Hemostatic Materials -A Preliminary Report in an Animal Model-

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Hemostasis is a key issue in surgery because uncontrolled surgical bleeding is associated with increased mortality rates and higher costs. Many types of topical hemostatic materials have been developed. One of the most current and most effective materials is TachoComb (TC), which is reabsorbed well by conversion into connective tissue. However, TC's excellent hemostatic effects are brought about by the use of hemostatic components and a framework derived from human and animal tissues. This means that TC use is associated with a risk of infectious diseases and allergic reactions.

To address the above-mentioned problems with the most current hemostatic material, we devised a new hemostatic material composed of a framework of a freeze-dried gelatin sponge combined with gelatin glue, thereby creating a new topical hemostatic material made of gelatin alone. The gelatin used in the material was prepared with alkali-treatment, and the immunogenicity and activity of viruses were eliminated almost completely. The framework absorbs blood flowing out from the wound immediately, activates autologous coagulation components in the absorbed blood and promotes blood coagulation at the bleeding site. The hemostatic effects of three types of hemostatic materials were compared, namely (A) the gelatin sponge combined with gelatin glue, (B) TC and (C) gelatin glue alone, using a dog's spleen with a shaved wound surface. Consequently, combined use of the gelatin sponge and gelatin glue exhibited significantly superior hemostatic effects to the other two materials. In the conclusion, the combination of a gelatin sponge sheet and gelatin glue is useful as a topical hemostatic material due to its superior hemostatic effects and extremely low risk of infectious diseases and allergic reactions.

**Key words:** topical hemostatic material, gelatin glue, gelatin sponge, TachoComb, spleen, hemostatic effect

## 1. Introduction

Hemostasis is a key issue in surgery because uncontrolled surgical bleeding is associated with

increased mortality rates and higher costs<sup>1,2)</sup>. Failure to achieve hemostasis can unnecessarily prolong the surgical procedure, impair wound healing, increase the risk of infection, and result in unanticipated exposure to

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blood products if the patient requires a transfusion<sup>1, 2)</sup>. A variety of hemostatic agents that mitigate uncontrolled bleeding are available, including topical hemostats, sealants and adhesives. Nevertheless, hemostasis is not achieved in as many as 40% of surgical patients because hemostatic agents are not used appropriately<sup>3)</sup>. Many types of topical hemostatic materials have been developed. Among these materials, one of the most useful and most current materials is TachoComb (TC). TC is a fibrin-coated collagen fleece that acts as a topical hemostatic agent and was originally developed for cardiac, thoracic and hepatic surgical use. TC possesses good adhesive properties and is reabsorbed well by conversion into connective tissue. Its hemostatic efficacy has been clinically assessed in conventional open liver, pancreatic, splenic, vascular, thoracic and gynecologic surgery<sup>4)</sup>. Although TC is indeed an excellent hemostatic material, its superb hemostatic effects are brought about by the use of hemostatic components and a framework derived from human and animal tissues such as fibrinogen from human blood and thrombin, aprotinin and collagen from other animals<sup>5)</sup>. This carries a risk of infectious diseases and allergic reactions because it is difficult to completely remove infectious contaminants and allergens in the manufacturing process of these hemostatic components<sup>6)</sup>.

To address the above-mentioned problems with the most current hemostatic material, we focused on (A) utilizing autologous blood-coagulating components with (B) a freeze-dried sponge of alkali-treated gelatin as a framework.<sup>7)</sup> Therefore, we devised a new topical hemostatic material made of alkali-treated gelatin alone. The immunogenicity and activity of viruses are eliminated almost completely through the gelatin alkali-treatment process. Moreover, freeze-dried alkaline-treated gelatin absorbs autologous blood-coagulating components and allows them to form a hemostatic gel with a framework of gelatin.

In the present animal experiment, we report that the use of a gelatin sponge combined with gelatin glue

prepared from alkali-treated gelatin exhibits superior hemostatic effects to TC, the most useful and most current hemostatic material.

## 2. Materials and Methods

### 2.1 Preparation of hemostatic materials

#### Usual tissue sealing sheet

TachoComb (CSL Behring, King of Prussia, PA, USA), which is composed of collagen, fibrinogen, thrombin and aprotinin, is one of the most widely used tissue-sealing sheets. TC was purchased from Wakenyaku Co. Ltd. (Osaka, Japan) and used in accordance with the manufacturer's instructions. TC was cut into square sheets measuring 30 mm × 30 mm in size just prior to use.

#### Gelatin sponge

Low-endotoxin gelatin extracted from porcine skins (type-I collagen, Medigelatin<sup>®</sup>) with an isoelectric point of 5 was supplied by Nippi Co. Ltd. (Tokyo, Japan). The gelatin was dissolved in distilled water to a concentration of 1.0 wt%. The 1.0 wt% gelatin solution was cast into a petri dish, frozen in a deep freezer (ULTRA LOW, SANYO, Osaka, Japan) at -80 °C for 30 minutes and freeze-dried for 24 hours in a vacuum freeze dryer (DRZ350WA, ADVANTEC, Tokyo, Japan) to create a gelatin sponge. After freeze-drying, the gelatin sponge sheet was removed from the petri dish and dehydrothermally cross-linked in a vacuum oven (DP41, Yamato Scientific Co., Ltd., Tokyo Japan) at 140 °C for three hours. The gelatin sponge sheet was cut into square sheets measuring 30 mm × 30 mm in size just prior to use.

#### Gelatin glue

The same gelatin dissolved in distilled water at 26 wt% was used as a gelatin solution. It was warmed to 45 °C before use. A glutaraldehyde solution at 25 wt% purchased from Sigma Aldrich (Japan) was diluted to 1 wt% with physiological saline. The gelatin and 1% glutaraldehyde (GA) solutions were stored separately in syringes. When the gelatin gel was applied onto the

wound surface, the gelatin and GA solutions were poured simultaneously at a ratio of 5 to 1 to react with each other and create a gelatin-gel.

## 2.2 Design of animal experiments

The animal experiments performed in this study were approved by the Doshisha University Animal Experimentation Committee. All animal care, housing and surgical and anesthetic procedures were performed in accordance with the Animal Care Guidelines of the Committee for Animal Research of Doshisha University and Nara Medical College.

Nine non-pregnant female Beagle dogs 2 years of age weighing 9.5-10.5 kg were purchased from Shimizu Laboratory Animal Supply Co. Ltd. (Kyoto, Japan). During the experimental period, all dogs were housed separately and maintained under standard conditions (a light-dark cycle of 12:12 hours, a mean temperature of 23 °C and a mean humidity of 50%). Standard laboratory dog chow and water were freely available. Before the study, the dogs were housed in the laboratory for one week. On the first experimental day, all dogs were assessed for the condition of their health.

## 2.3 Surgical technique used to evaluate the hemostat on the spleen

All surgeries were performed under sterile conditions, and all procedures were completed by one team of three persons. The dogs were given intravenous anesthesia at a dose of 34 sodium pentobarbital (Somnopenyl<sup>®</sup>, Kyoritsu Seiyaku, Tokyo, Japan) mg/kg of body weight with a syringe and a 23G injection needle. Under the above-described general anesthesia, the dogs were fixed in the dorsal position. The hair on the abdomen was cut, and the skin was cleaned with a solution of 5% chlorohexidine + 80% ethanol and sterilized with 10% povidone iodine solution. In all of the dogs, a 12 cm long midline upper abdominal incision was made. The surface of the upper or lower part of the spleen was shaved with scissors to 1-2 mm in depth and 20 mm×10 mm in area to allow the shaved wound to bleed diffusely. TC, gelatin glue alone or gelatin sponge

plus gelatin glue was applied at random to the bleeding site of either the upper or lower part of the spleen. This experiment was repeated in 6 experiments for each hemostatic material.

When TC was applied, after wiping the blood from the shaved surface of the spleen with a gauze, a sheet of TC was tightly placed over the shaved wound surface. Then, the TC sheet was covered by gauze and compressed onto the shaved surface over the gauze with finger for one minute. The gauze was then gently removed to allow rebleeding during an observation period of the next five minutes (totaling six minutes after placement of the hemostatic material).

When gelatin glue alone was applied, after wiping the blood from the shaved wound surface with a gauze, the wound surface was compressed for one minute with fingers over the gauze. The gauze was then gently removed, and 2.0 ml of the gelatin solution and 0.20 ml of the GA solution were poured simultaneously over the shaved wound surface to allow for gelatin-gel bonding and sealing over the wound surface.

When gelatin sponge was applied with gelatin glue, after wiping the blood from the shaved wound surface with gauze, the gelatin sponge sheet was placed over the shaved wound surface and the gelatin sponge was compressed for one minute with fingers over the gauze. The gauze was then gently removed, and 2.0 ml of the gelatin solution and 0.20 ml of the GA solution were poured simultaneously over the gelatin sponge.

The hemostatic effects were evaluated during an observation period of five minutes (timing 1 to timing 3 in Figure1) because the standard range in the clinical test of Duke's method is one to five minutes in bleeding time<sup>8)</sup>. When blood was seen flowing out during the observation period of five minutes, the hemostasis was evaluated to be broken (rebleeding) and the time-point of re-bleeding was recorded.

The hemostatic materials were also evaluated for usability in reference to handling during surgery.

## 2.4 Statistical analysis

The data are expressed as the mean  $\pm$  standard deviation. The statistical analyses were carried out using the Chi-square test with the software program Stat/Mate III, Windows Version (ATMS Co., Tokyo, Japan). Differences were considered to be statistically significant when the p value was less than 0.05.

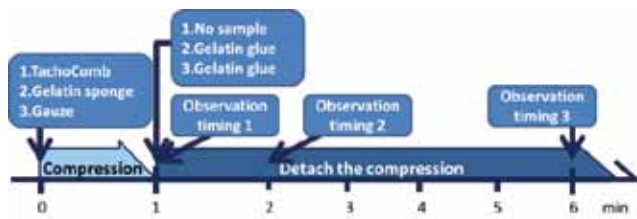


Fig. 1. Schematic diagram of the hemostatic experiment.

### 3. Results

Table 1. Hemostatic effects.

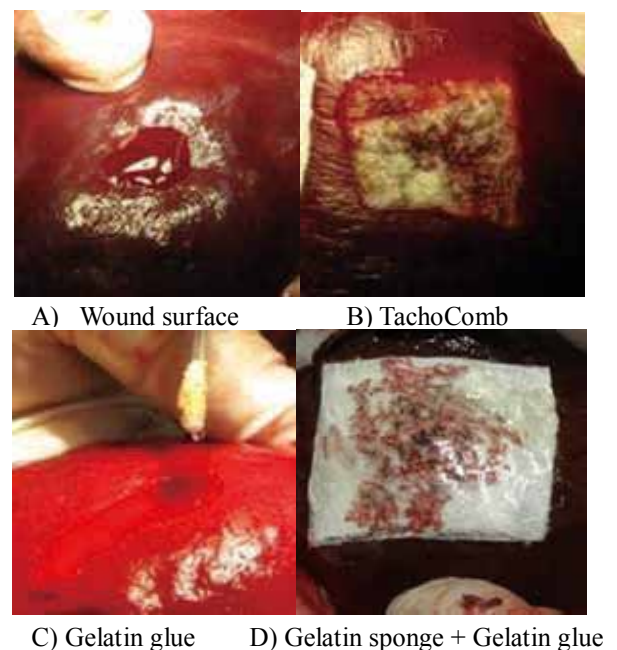
Hemostatic groups	Number of experiment	Bleeding-positive at the observation time			Totals of bleeding-positives	Totals of bleeding-negatives
		Observation timing 1	Observation timing 2	Observation timing 3		
Gelatin sponge + Gelatin glue group	6	0	0	0	0	6 *
TC group	6	0	2	3	5	1 **
Gelatin glue alone group	6	6	0	0	6	0 ***

\*~\*\* P<0.01 \*~\*\*\* P<0.001 \*\*~\*\*\* NS

Table 1 shows the hemostatic results observed in the TC group, the gelatin glue alone group and the gelatin glue + gelatin sponge group. There were significant differences between the gelatin sponge + gelatin glue group and the gelatin glue alone group ( $p<0.001$ ) and between the gelatin sponge + gelatin glue group and the TC group ( $p<0.01$ ), although there were no differences between the gelatin glue alone group and the TC group.

In the gelatin glue alone group, the bleeding blood flow washed out the gelatin glue before the glue turned into gel and adhered to the wound surface. Therefore, when the glue alone was used against the bleeding wound surface, there was a poor hemostatic effect.

The gelatin sponge + gelatin glue group exhibited superior hemostatic effects not only with the gelatin glue alone, but also with TC. After being compressed for one minute onto the bleeding surface, even before the gelatin glue was combined with the gelatin sponge, the gelatin sponge exhibited good adhesiveness because the gelatin sponge layer turned into an adherent gel layer after absorbing blood. Then, the gelatin glue was added over the gelatin sponge and the sponge was tightly fixed onto the shaved wound surface, with stopping the bleeding. Therefore, the combined use of the gelatin sponge and gelatin glue exhibited remarkable hemostatic effects against diffuse bleeding.



#### 4. Discussion

The combination of the gelatin sponge and gelatin glue was most effective in hemostasis compared with not only gelatin glue alone, but also TC, which is the most useful and most current hemostatic material. This can be explained by two reasons. First, after being placed onto the bleeding wound surface, the gelatin sponge layer smoothly absorbs blood and activates autologous blood-coagulating components in the blood. Then, the components create the framework of the gelatin sponge by absorbing blood and creating an adherent gel layer with hemostatic activity. Therefore, the gel layer in the gelatin sponge changes in adhesiveness and hemostatic activity due to the activated autologous hemostatic components. Second, the gelatin glue is added over the gelatin sponge, and the sponge layer is tightly fixed onto the organ surface.

The results of the hemostatic experiment suggest that gelatin glue should be combined with a sheet of gelatin sponge to reinforce the fixation of the sponge sheet onto the organ surface, rather than pouring gelatin glue alone onto the bleeding site.

Although TC is indeed an excellent hemostatic material, it is composed of hemostatic components and a framework derived from human and animal tissues, which is associated with a risk of infectious diseases and allergic reactions.

The gelatin sponge and gelatin glue are made of alkaline-treated gelatin, which hardly carries risks of allergic reactions or infectious diseases since the gelatin alkali-treatment process eliminates the immunogenicity and activity of viruses almost completely.<sup>7)</sup> The hemostatic effects are actualized by the autologous hemostatic components and framework of the freeze-dried gelatin sponge. Therefore, the combination of the gelatin sponge sheet and gelatin glue is associated with an extremely low risk of infectious diseases and allergic reactions.

In conclusion, the combination of a gelatin sponge sheet and gelatin glue is useful as a topical hemostatic

material due to its superior hemostatic effects and extremely low risk of infectious diseases and allergic reactions.

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