Chitosan Sponge Has a Better Local Hemostatic Effect Than Alginate Sponge in Animal Experiment

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Collagen-preparation and fibrin glue are widely used local hemostatic materials. Collagen-preparation, however, can promote local infections, and tends to cause allergic reactions, since it contains proteins from different kinds of animals. Fibrin glue is associated with risks of viral infections, because it contains materials from human beings. Therefore, this laboratory developed the "chitosan sponge", as a novel local hemostatic material with few such problems, because chitosan is derived from neither different kinds of animals nor humans but from crabs. In the experiment, chitosan sponge was made by a freeze-drying method, and was evaluated in for tissue-adhesion intensity and hemostatic effects, in comparison to sponge of alginate, another hemostatic material. The tissue-adhesion intensity of these materials was evaluated by their maximal shear stress. The maximal shear stress in the chitosan group was significantly large in comparison to that in the alginate group. The hemostatic effect was indicated with blood amount bleeding from the mesentery in rats. There was significantly little bleeding in the chitosan group in comparison to that in the alginate group. These results suggest that the chitosan sponge can be useful as a novel local hemostatic material.

Key words: hemostatic material, chitosan sponge, alginate sponge, hemostatic effect, tissue-adhesion intensity

1. Introduction

Sufficient and quick hemostasis is required when serious bleeding takes place during an operation. Therefore, hemostasis is very important during a surgery. Collagen-preparation [1] and fibrin glue [2] are widely used as local hemostatic materials for such a purpose. Collagen-preparation, however, can promote local infections, and require careful observation after use. In addition, the foreign proteins may induce allergic reactions such as anaphylaxis, since it originates from animals [3]. Fibrin glue is associated with risks of serious infections such as hepatitis virus and human immunodeficiency virus (HIV), since it is derived from human blood [4]. Therefore, there are problems associated with the available hemostatic materials.

A novel local hemostatic material has been developed using chitosan derived from crabs [5], with a

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proven hemostatic effect and few such risks [6]. The chitosan sponge has better hemostatic effects than fibrin glue [5]. This study will show that chitosan sponge has superior tissue-adhesion intensity and hemostatic effect to sponge of alginate, which is another recently developed hemostatic material that is not derived from animals [7].

2. Materials and Methods

2.1 *Preparation of hemostatic materials*

A 3 g sample of Chitosan (Kimica Chitosan, Kimica Corporation, Tokyo, Japan) was dissolved in 300 ml distilled water (Otsuka Distilled Water, Otsuka Pharmaceutical Co., Ltd, Tokyo, Japan), and then, 1.5 ml of 99.7% acetic acid (Acetic Acid, Wako Pure Chemical Industries, Ltd, Osaka, Japan) was added in order to dissolve chitosan, resulting in a 0.01 g/ml of chitosan solution. This solution was poured into a petri dish, and then, frozen at −80ºC for 3 days, then freeze-dried at a pressure of less than 13.3 Pa for 4 days with a freeze-dryer (FZ-2.5, Asahi Life Science Co., Ltd, Saitama, Japan) until a sponge-like appearance was attained.

A sample of 4 g of Sodium alginate (Sodium Alginate, Wako Pure Chemical Industries, Ltd, Osaka, Japan) was dissolved in 400 ml distilled water, resulting in a 0.01 g/ml alginate solution. This solution was poured into a petri dish, and then, frozen at −80ºC for 3 days, then freeze-dried at a pressure of less than 13.3 Pa for 4 days in the freeze-dryer until a sponge-like appearance was attained.

Therefore, the each hemostatic material was made into a sponge. The sponge plates were cut into a 2.0 cm×2.0 cm square-shaped sheet that weighed was 0.03 g.

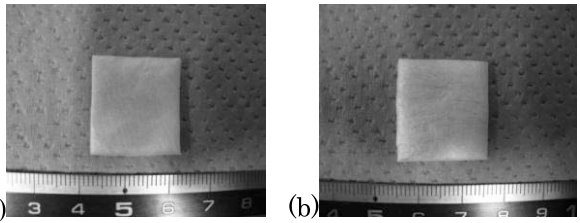

Fig. 1. Photographs show the square-shaped sheets of (a) chitosan sponge and (b) alginate sponge.

2.2 *Ex vivo examination*

2.2.1 *Shear force between the hemostatic material and porcine skins*

Fresh porcine skins were obtained from a local slaughterhouse. The porcine skins were thoroughly washed, and shaved finely. They were cut into a 2.0 cm×2.0 cm square skin, and the fatty layers of the skin were removed. The epidermal side of the porcine skin was firmly fixed to a fixed board by cyanoacrylate. One square-shaped sheet of chitosan sponge or alginate sponge was placed between two dermal sides of the porcine skins, and a load of 50 g/cm^2 was applied for two min. The shear force was measured using a tensile machine (CPU gauge: MODEL-9500, TEST STAND: MODEL-1356, Aikoh Engineering Co., Ltd, Osaka, Japan) at a separation rate of 80 mm/min. The maximal shear force was determined as the maximal load at the time when the square-shaped sheet of chitosan sponge or alginate sponge was divided from the porcine skin. The measurement was performed 6 times. The data from all 6 attempts were used for the evaluation in the alginate group. However, the data from only 5 attempts was used in the chitosan group, because the fixation between the porcine skin and the fixed board came loose in one of the 6 measurements, and the result was excluded. The measured result was presented as the maximal shear stress (gf/cm^2).

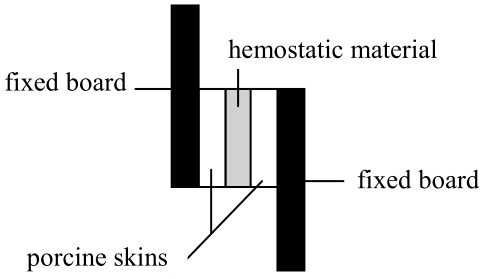

Fig. 2. Shear force

2.3 *In vivo examination*

2.3.1 *Experimental design and animal protocol*

The animal experiments were approved by Doshisha University Animal Experimentation Committee. All the surgical procedures and anesthesia were performed in accordance with the animal care guidelines of Doshisha University.

Sixteen 8-10-week-old female Wistar/ST rats and weighing 180-230 g were used in this study. All the rats were maintained under specific pathogen free circumstances, at room temperature (21-25℃), humidity of 45-65%, and on a 7AM to 7PM light schedule. The rats were fed with standard pellet diets, and allowed tap water *ad libitum*. The rats were housed in the laboratory for one week before the study. The rats' health was checked, and they were randomly assigned into two groups of 8 rats each: the chitosan group and alginate group.

2.3.2 *Surgical technique*

All operations were performed under sterile conditions. The rats were given isoflurane (Escain®, Mylan, Inc., Osaka, Japan) inhalation anesthesia. Sodium pentobarbital (4.5 mg, Sommnopentyl®, Kyoritsu Seiyaku, Inc., Tokyo, Japan) was dissolved in one ml of physiological saline. One ml of pentobarbital aqueous solution was administrated intraperitoneally to every rat using a tuberculin syringe and a 23G injection needle.

The anesthetized rats were fixed in the supine position, and underwent a laparotomy with a median incision. The mesentery was exposed, and a parafilm sheet was laid under the mesentery. The primary branch of the mesenteric blood vessel was cut with scissors, and the bleeding point was put between two square-shaped sheets of chitosan sponge or alginate sponge. The bleeding point was compressed at 160 g with a finger over the square-shaped sheets of the hemostatic material for 5 minutes to stop the bleeding. The 4 square-shaped sheets of the hemostatic material and gauze including blood we weighed to evaluate the amount of bleeding. The difference between the total weight of the 4 square-shaped sheets and the gauze before the experiment of the hemostasis and after the same experiment was defined as the amount of bleeding.

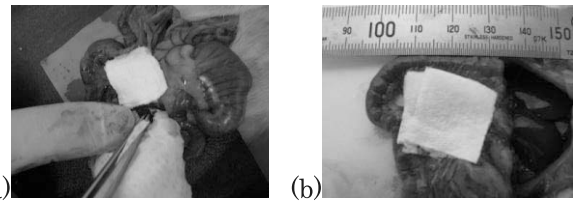

Fig. 3. Photographs (a) the square-shaped sheets of chitosan sponge and (b) alginate sponge which are placed on the mesentery for hemostasis.

2.4 *Statistical Analysis*

The measured results are presented as the mean±standard deviation (SD), and then, difference was determined by Student's *t*-test. A P value of less than 0.05 was considered to be significant statistically.

3. Results

3.1 *Ex vivo examination*

3.1.1 *Maximal shear stress of chitosan and alginate sponges*

The results of the maximal shear stress test in the chitosan and the alginate groups are displayed in Figure 4. The maximal shear stress in the chitosan group and the alginate group were 139.75±12.309 and 64.83±28.768 gf/cm^2, respectively. The maximal shear stress in the chitosan group was significantly ($p<0.01$) large in comparison to that in the alginate group.

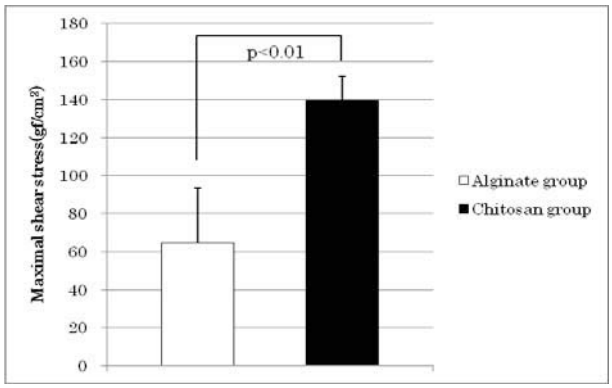

Fig. 4. Maximal shear stress in the chitosan and the alginate groups. The column indicates the mean, and the bar shows SD.

3.2 *In vivo examination*

3.2.1 *Hemostatic effect of chitosan and alginate sponges*

The hemostatic results in the chitosan and the alginate groups are displayed in Figure 5. The amount of bleeding in the chitosan group and the alginate group were 0.195±0.080 and 1.589±0.315 g, respectively. The amount of bleeding in the chitosan group was significantly ($p<0.01$) small in comparison to that in the alginate group.

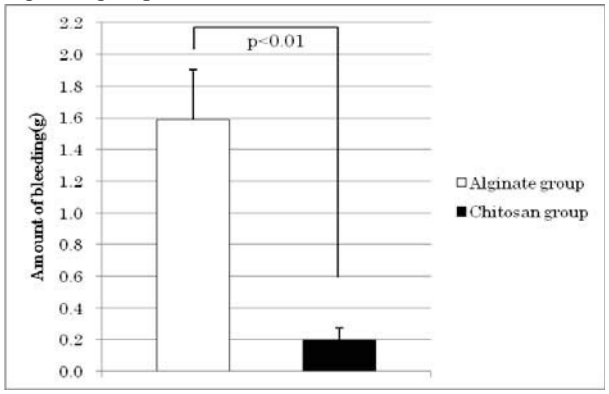

Fig. 5. Amount of bleeding in the chitosan and the alginate groups. The column indicates the mean, and the bar shows SD.

4. Discussion

This experiment used chitosan as a novel local hemostatic material. Chitosan is a polysaccharide containing partially N-deacetylated chitin, which is a linear homopolymer of 1,4-β-linked N-acetyl-D-glucosamine [8]. The chitosan was used in this experiment because of its hemostatic effect. The positive charged amino groups in the molecule of chitosan draw the blood platelets with a negative surface charged, and promote a platelet aggregation action [5].

Chitosan was prepared in the form of sponges rather than other forms, because other forms of hemostatic materials have the following problems. First, it is difficult to handle collagen powder for hemostasis during a surgery. It adheres to the wet glove surface, tweezers and sites other than the hemostatic region [3]. Moreover, histological findings reveal that alginate powder activates foreign-body reactions, because alginate powder is hard to dissolve in water, and remains in the powder-form on the surface of tissues for a long time [9]. Second, a sheet is too hard to fit onto the bleeding surface of the organs although handling a sheet is simpler than a powder [3]. Third, spraying a liquid hemostatic agent does not adhere to a glove, and it is the simplest to handle. However, it is difficult for the surgeon to target the bleeding site and have it adhere correctly there [3]. Chitosan was thought to have these problems similarly when it is made into the hemostatic material. Therefore, chitosan was made into the form of sponge. In fact, the chitosan sponge absorbs blood smoothly, fits well to the bleeding surface, and stops the bleeding when the surgeon presses it with his/her fingers from above, as the standard hemostatic maneuver.

Sodium alginate was chosen as the control to compare with the chitosan sponge in this experiment. Alginate is an anionic polysaccharide containing β-D-mannuronate and its C5 epimer, α-L-glucuronate [8]. The hemostatic effect of sodium alginate involve adhesion and covering the bleeding site, promotion of fibrin formation and a wound-healing facilitatory effect [7]. A 5% solution of sodium alginate has beneficial effect on gastrointestinal bleeding or reflux esophagitis [7]. Therefore, alginate is a recently developed hemostatic material.

The current experiment showed that the amount of bleeding in the chitosan group was significantly smaller than that in the alginate group. This result suggests that the chitosan sponge has a superior hemostatic effect than the alginate sponge.

Strong adhesion is one of the major factors that contribute to a strong hemostatic effect [4]. Therefore, in this experiment, the tissue-adhesion was also compared by measuring the maximal shear stress of the chitosan sponge in comparison to the alginate sponge. The results

showed that the maximal shear stress in the chitosan group was significantly greater than that in the alginate group. This suggests that chitosan may provide a higher-performance hemostatic effect due to stronger tissue-adhesion than the alginate sponge.

Chitosan has other characteristics that distinguish it from collagen or fibrin, which are the common hemostatic materials. Chitosan is composed of a polysaccharide, and is thought to have very low risks of allergic reactions in comparison to collagen composed of foreign proteins [3), 6), 8)]. The chitosan in the current experiment originated from crabs in the crustacean, thus the risks of viral infections are also much lower than that with collagen originating from mammals [3), 5), 6)]. Fibrin glue, the other commonly used hemostatic material, also has risks of passing on infections by human pathogenic agents, since it is of human origin [4)].

In the conclusion, a chitosan sponge has a stronger hemostatic effect with stronger adhesion to the tissue surface in comparison to an alginate sponge.

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References

1) K. Tsuchiya, “A novel usage of topical hemostats (microfibrillar collagen : Avitene®) in spinal surgery”, Pharma Medica, 24, 126-128 (2006) (in Japanese)
2) Y. Ikeda, K. Sugimachi, S. Oka, M. Tomikawa, K. Yagi, S. Tsukamoto, K. Kawasaki, S. Yamamura, D. Korenaga, and K. Takenaka, “Combined used of liquid fibrin adhesive and polyglycolic acid felt for hemorrhage prevention in liver section”, The Japanese Journal of Clinical and Experimental Medicine, 84, 148-152 (2007) (in Japanese)
3) K. Tsuchiya, K. Yamaoka, M. Miyagi, and T. Okamura, “Consideration of topical hemostats in spinal surgery : A novel usage of microfibrillar collagen”, Orthopedics and Traumatology, 56, 664-668 (2007) (in Japanese)
4) Y. Murakami, “Can sea adhesion protein be used for a surgical operation? ~New development of the biomaterial for hemostasis~”, Experimental Medicine, 26, 900-901 (2008) (in Japanese)
5) A. Hashimoto, Y. Hirasaki, H. Tsujimoto, and A. Hagiwara, “Development of novel infectious agent free-locally administered hemostatic material made from chitosan”, The Science and Engineering Review of Doshisha University, 50, 82-87 (2009) (in Japanese with English abstract)
6) T. Horio, M. Ishihara, M. Fujita, S. Kishimoto, Y. Kanatani, T. Ishizuka, Y. Nogami, S. Nakamura, Y. Tanaka, Y. Morimoto, and T. Maehara, “Effect of photocrosslinkable chitosan hydrogel and its sponges to stop bleeding in a rat liver injury model”, Artificial Organs, 34, 342-347 (2010)
7) M. Ueda, M. Ueki, K. Torii, and S. Noda, “Topical styptic in cervical cancer examination. Effectiveness of sodium alginate (align).”, Obstet. Gynecol., 63, 1457-1463 (1996) (in Japanese)
8) H. Hattori, Y. Amano, Y. Nogami, B. Takase, and M. Ishihara, “Hemostasis for severe hemorrhage with photocrosslinkable chitosan hydrogel and calcium alginate”, Annals of Biomedical Engineering, 38, 3724-3732 (2010)
9) Y. Hirasaki, M. Fukunaga, A. Kidokoro, A. Hashimoto, T. Nakamura, H. Tsujimoto, and A. Hagiwara, “Development of a novel antiadhesive material, alginate flakes, *ex vivo* and *in vivo*”, Surgery Today, 41, 970-977 (2011)