

Comparison of the Wound Healing Effect of Cellulose and Gelatin: An *In Vivo* Study

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Background Many topical hemostatics are widely applied for bleeding control. They can be classified into two categories according to their mechanism of action on the clotting cascade in a biologically active or passive manner. Passive hemostatics include cellulose and gelatin. We performed an experimental study to compare the effect of passive hemostatics in wound healing by applying them to a rectus abdominis muscle defect of white mice.

Methods Surgicel is a sterile absorbable knitted fabric prepared by the controlled oxidation of regenerated cellulose. Spongostan is an absorbable hemostatic gelatin sponge. In 30 mice, a 1 × 1 cm defect was created on the rectus abdominis muscle and the materials were applied in three ways: control group, cellulose (Surgicel) group, gelatin (Spongostan) group. For the histologic analysis, biopsies were performed at 3 and 28 days.

Results After 3 days, the cellulose group showed limited granulation formation with acute inflammatory reactions similar to the control group. At the 28th day, moderate amounts of granulation tissue formation was observed with milder inflammatory reactions than the control group. In the gelatin group, after 3 days, gelatin remnants were observed surrounded by severe inflammatory changes. After 28 days, the same quantity of gelatin remnants could be still observed.

Conclusions This study suggests that cellulose is associated with minimal morbidity in wound healing, while the use of gelatin shows severe adverse tissue reactions with delayed wound healing. Consequently, cellulose is better than gelatin when considering wound healing.

Keywords Cellulose / Gelatin / Hemostatics / Wound healing

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INTRODUCTION

Nowadays, many kinds of hemostatics are used in various surgical fields. Generally, in the choice of hemostatics, how quickly and how effectively it works are important factors. For this reason, many hemostatic studies have been performed to compare hemostatic effects *in vivo* and *in vitro* [1-4]. However, it is controversial how these hemostatics affect wound healing. For their proper use, study on the effect of hemostatics on wound healing

is necessary. In this study, we performed an experimental study to compare the effects on wound healing of two widely-used passive hemostatics (cellulose, Surgicel; gelatin, Spongostan) that have been proven to control bleeding arrest. They were applied to rectus abdominis muscle defects of white mice and histologic analysis was performed. The goal was to inform the choice of hemostatics by providing information on the hemostatics' effect on wound healing.

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METHODS

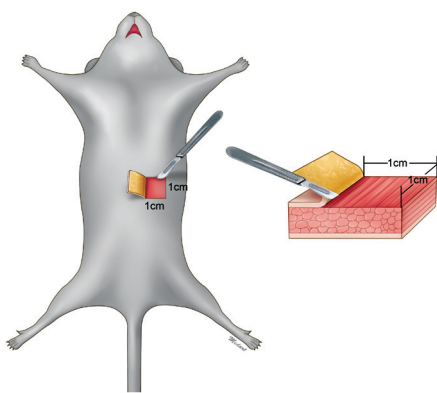
Materials

Experimental animals

Thirty 8-week-old, 280 to 300 g, white male mice from the ICR

Fig. 1. Anatomical drawing of a wound-induced rat

A No.15 scalpel was used for incision at the abdomen and a 1 × 1 cm full layer muscle defect was made in the left rectus abdominis muscle. Cellulose or gelatin was then applied to each defect site. Each abdominal wound was sutured with 6-0 nylon and daily wound dressing was performed with a 10% povidone-iodine and 70% alcohol solution.



(CrjBgi: CD1) strain living in the same conditions were used in the study. After a 1 week period of adjustment in the laboratory, the study was begun.

Experimental material

1) Cellulose (Surgicel, absorbable oxidized regenerated cellulose, Johnson & Johnson, Arlington, TX, USA). 2) Gelatin (Spongostan, absorbable hemostatic gelatin sponge, Johnson & Johnson).

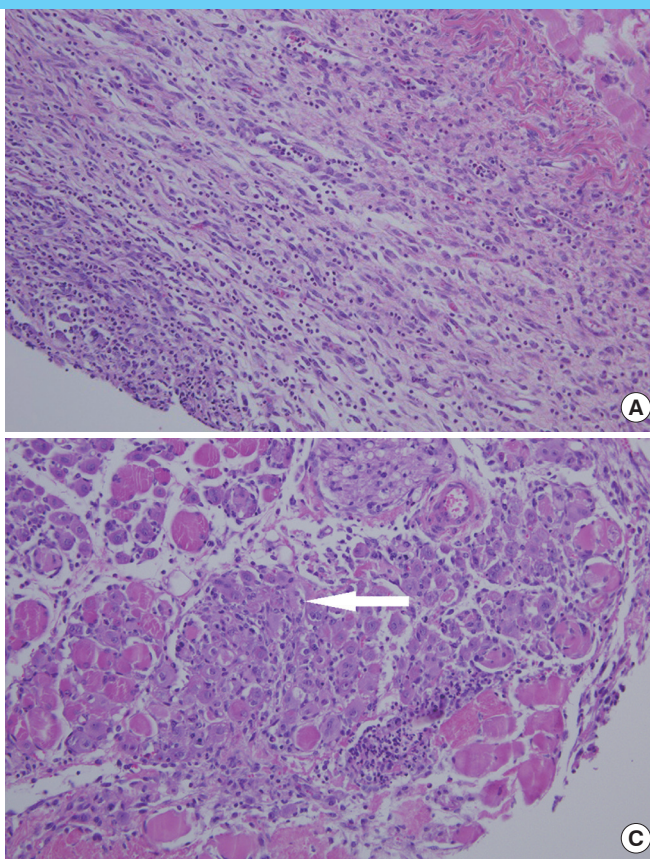
Methods

Wound induction

40 mg/kg of pentobarbital sodium (Hanlim Pharmaceutical, Seoul, Korea) and 20 mg/kg of ketamine hydrochloride (Ketar) (Yuhan, Seoul, Korea) were injected into the intraperitoneal cavity of white mice for anesthesia. We removed the abdominal hair and used a 10% povidone-iodine and 70% alcohol solution for disinfection and then made an incision with a No.15 scalpel in the abdomen and made a 1 × 1 cm full layer muscle defect in the left rectus abdominis muscle by separating the subcutaneous fat (Fig. 1).

Fig. 2. Three days after inducing the wound (H&E, ×200)

(A) Control group. (B) Cellulose group, acute inflammatory cells infiltrated the tissue (white arrow) similarly to the control group. (C) Gelatin group, focal inflammatory cells infiltrated (white arrow) the tissue.



Wound care

30 white mice were randomly classified to 3 groups, each composed of 10 mice. Control group, no care was provided for the defects; cellulose group, the muscle defect site was treated with cellulose (Surgicel); gelatin group, the muscle defect site was treated with gelatin (Spongostan). The cellulose or gelatin was applied in almost the same volume to each animal. The abdominal wound was sutured with 6-0 nylon and dressing was done with 10% povidone-iodine and 70% alcohol solution. The mice were caged, one mouse in one cage, and observed for 28 days. During the experiment, feed (5L79, PMI Inc., St. Louis, MO, USA) and water was provided ad libitum.

Wound biopsy

On postoperative days 3 and 28, we selected 5 mice from each group and took tissue, including wound site. The tissue taken from each mouse was fixed with 10% formalin solution for more than 6 hours, and stained with hematoxylin and eosin stain. We observed any proliferation of granulation tissue, the degree of inflammation, and the presence or absence of foreign bodies, and we measured the degree of wound healing in each group by comparing it with the control group.

RESULTS

We compared the wound healing effect of each of the two hemostatics by observing any proliferation of granulation tissue, the degree of inflammation, and the presence or absence of giant cells and foreign bodies. Each groups showed similar histologic findings.

Wound biopsy

Cellulose group

Three days after inducing the wounds, similarly to the control group, proliferation of granulation tissue was rarely found and acute inflammatory cells had infiltrated the tissue (Fig. 2).

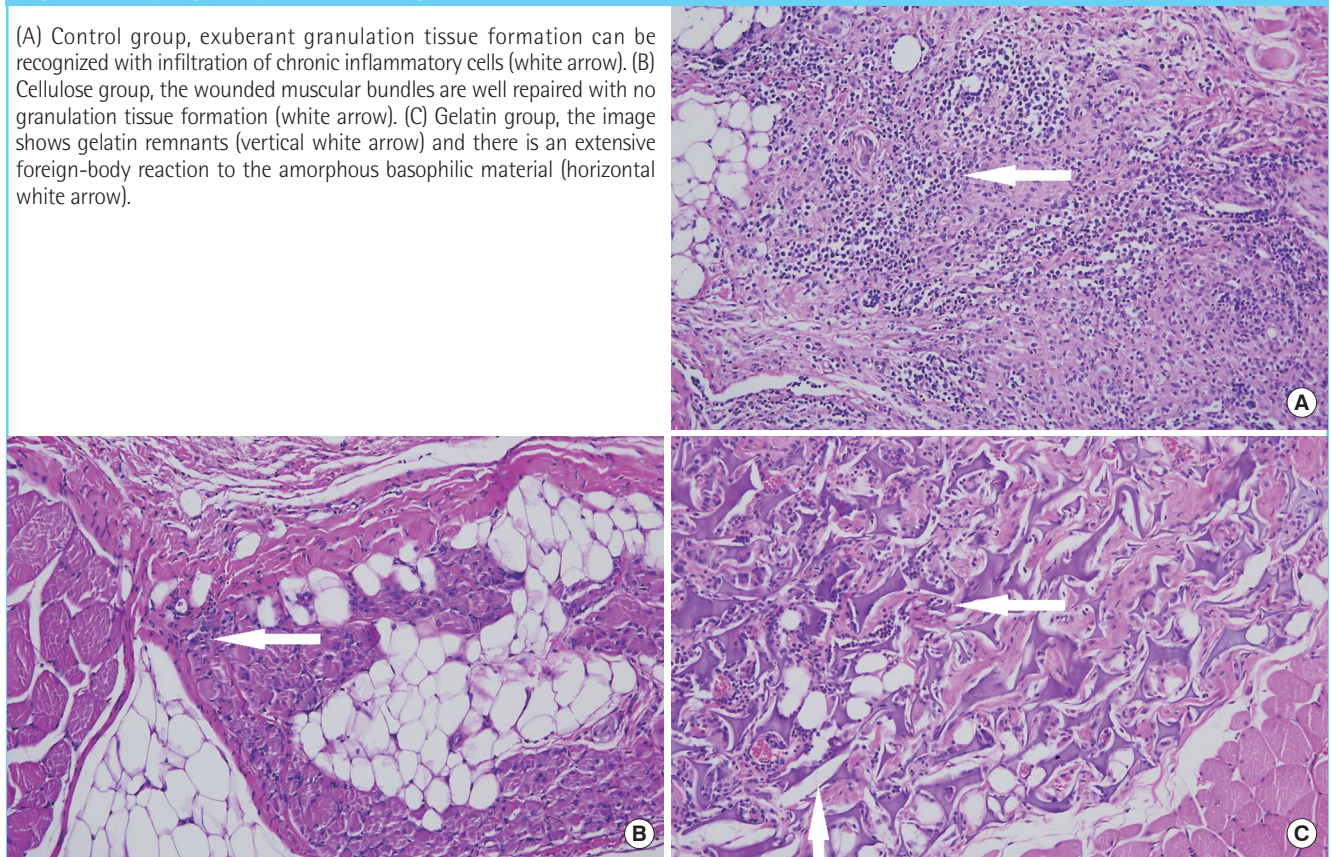
Twenty-eight days after inducing the wounds, we could not find cellulose debris. Compared to the control group, the proliferation of granulation tissue was more severe while the signs of inflammation were lower (Fig. 3). As a result, cellulose did not delay the wound healing compared with the control group.

Gelatin group

Three days after inducing wound, proliferation of granulation tissue is rarely found and focal inflammatory cell is infiltrated to tissue (Fig. 2).

Fig. 3. Twenty-eight days after inducing the wound (H&E, ×200)

(A) Control group, exuberant granulation tissue formation can be recognized with infiltration of chronic inflammatory cells (white arrow). (B) Cellulose group, the wounded muscular bundles are well repaired with no granulation tissue formation (white arrow). (C) Gelatin group, the image shows gelatin remnants (vertical white arrow) and there is an extensive foreign-body reaction to the amorphous basophilic material (horizontal white arrow).



Twenty-eight days after inducing the wounds, the debris of gelatin was found, and giant cells and modified macrophages around the debris are found. Compared to the control group, there was less proliferation of granulation tissue and there was much more inflammation and a greater foreign body reaction (Fig. 3). As a result, gelatin was found to delay wound healing.

DISCUSSION

Wound healing generally goes through an inflammation phase, epithelial phase, proliferation phase, and maturation phase [5]. If the inflammation phase continues for a long time, the next phase is delayed [5]. For this reason, inflammation phase is the most important of the 4 phases. In the inflammatory phase, as endothelial cells of normal vessels are damaged, thromboplastin is secreted, causing the formation of a thrombus and factor VII, which in turn causes platelet aggregation. As collagen that is under endothelial cell is exposed, the platelets adhere to the collagen [5]. This process helps hemostasis. Then the next phase begins only after the debris of damaged tissue, foreign bodies, and necrotized tissue are cleaned and removed by acute and chronic inflammatory cells [5]. Therefore, the goal of wound healing is the early triggering of hemostasis and accelerating the transition to the next step after the inflammatory phase.

When methods such as compression, suture, and electrocautery are not effective or cannot be performed for hemostasis in primary wound care, hemostatics can be helpful. Hemostatics can be classified into two types; one type including cellulose, gelatin, and collagen acts as a physical structure within which platelets can aggregate and stops bleeding indirectly; another type, such as thrombin, acts directly on the last step of the coagulation pathway [6]. These hemostatics prevent hemorrhage, decrease the use of systemic coagulants, help reducing surgical time, and contribute to a patient's quick recovery [7]. However, the remnants can induce a foreign body reaction, chronic inflammation, or infection leading to formation of granuloma and interfere with the wound healing process [8]. The authors compared the wound healing effect of two widely used hemostatics because the wound healing effect of hemostatics is controversial and making the choice of which hemostatic to use is difficult. The hemostatics used in the study were Surgicel composed of cellulose and Spongostan composed of gelatin. These hemostatics act indirectly on hemostasis.

Surgicel, a knitted fabric prepared by the controlled oxidation of regenerated cellulose, is a light/pale yellow color sterile gauze-type absorbable hemostatic. It is clinically effective as an adjunct for capillary and venous hemorrhage in various fields of operation, and is absorbed without leaving any foreign body after

being used for wound hemostasis in peeling, tissue biopsy, nail avulsion, and trauma [9,10]. Moreover, there is a report of the antibiotic effect after the use of Surgicel *in vivo*, and growth of antibiotic resistant strains were also found to be effectively suppressed [11,12]. Even though Surgicel is widely used in wounds that need hemostasis, its wound healing effect is still controversial. The present study's results of comparing the tissue biopsy of the cellulose group with the control group showed that there is no debris or foreign body and the experimental group shifted earlier from the inflammation phase to the next phase. Results also showed that cellulose does not delay wound healing since there was no difference in granulation tissue growth between the experimental and control groups.

Spongostan, composed of a gelatin ingredient, is a hemostatic in the form of an absorbant sponge. It has been reported that Spongostan is effective in hemostasis [13]. It is used at the site of extraction of a tooth or in the donor site of bone graft [2]. However, recently, it has been reported that it delays recovery in the region of bone biopsy [2,4]. According to our study, the debris of Spongostan caused a chronic foreign body reaction. Compared to the control group, inflammation was more severe and there was less granulation tissue formation. Recovery was delayed, too. It seems that this result proceeded from the difference in the mechanism of absorption between the cellulose and gelatin. Spongostan, composed of gelatin, is decomposed by foreign body giant cells in animal studies. Cytoplasmic projections and lysosomes participate in this phagocytosis process and more than 6 weeks is needed for the remnants of Spongostan to be completely removed [14]. On the other hand, Surgicel, composed of cellulose, which can be decomposed to glucose, is in an easily absorbable form, by cellulose in the body, the remnants of which remain more briefly than those of Spongostan [15].

The results from our study showed that cellulose, compared to gelatin, does not hinder the wound healing process as much. However, to understand the clinical significance of this finding, factors such as the degree of granulation tissue proliferation and the degree of necrosis and inflammation should have been quantified to compare the wound healing effect.

This study compared the wound healing effect of cellulose and gelatin, agents known to be effective in bleeding control, with the control group by applying each of them to mice with muscle defects. The results showed that gelatin, compared to cellulose, greatly delayed the wound healing. Therefore, when hemostatics are needed, cellulose, instead of gelatin, is recommended, considering its effects on wound healing.

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Discussion**Comparison of Wound Healing Effect of Cellulose and Gelatin: An *In Vivo* Study**

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I have read this paper interestingly. This topic is enough to arouse reader's interest.

After the bleeding is discontinued after applying hemostatic, hemostatic will be absorbed slowly. However during the absorption, it should not hinder wound healing. Therefore the comparison of hemostatic effect on wound healing seems to be important when surgeon choose the hemostatic. Therefore the purpose of this paper is valuable for publication.

However there are some shortcomings in this study. First, author's follow-up period was only 4 weeks. However, the gelatin is known to be usually absorbed after at least 6 weeks according to previous study [1]. Naturally remnant gelatin cause an inflammation, and reversely the inflammation is necessary for the gelatin to be absorbed. Therefore the follow up period should be more longer than at least 6 weeks.

Second, the model of wound healing do not seem to be proper.

The authors use the muscle defect model among various wound healing models such as skin defect model, bone defect model, cartilage defect model, etc. The reason why the authors chose should be clarified. Moreover I think that the author should investigate the final results of wound healing such as the regeneration of muscle or its function.

Third, they evaluated the results not quantitatively, but qualitatively. Therefore the results do not seem to be scientific and it seems anecdotal.

Although there are above mentioned shortcomings, I would like to appreciate the authors's effort to make this study.

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