Development and Evaluation of Thrombin-Loaded Gelatin Hemostatic Sheets for Spinal Surgery Applications

Atsuyuki Kawabata[1)], Satoru Egawa[1)], Makoto Ogino[2)] and Toshitaka Yoshii[1)]

1) Department of Orthopedic Surgery, Tokyo Medical and Dental University, Tokyo, Japan
2) Astellas Pharma Inc. Drug Discovery Research, Tokyo, Japan

Abstract:

Introduction: During spinal surgery, management of intraoperative bleeding and effective hemostasis are required to clearly visualize the surgical field and to safely perform procedures and positive postoperative outcomes. However, it is challenging to stop bleeding from the venous plexus around the dural sac due to the potential risk of neural tissue damage. We aimed to develop hemostatic sheets with appropriate characteristics for spinal surgery, such as softness, appropriate thickness, biodegradability, thrombin bioactivity, and minimal water-induced expansion.

Methods: Hemostatic sheets were made by dissolving bovine bone-derived gelatin in water and aerating it to form foam, followed by freeze-drying, crosslinking, and thrombin-soaking. Sheets A to H were produced with different gelatin concentrations, foam densities, and crosslinking times by additional heat treatment. The sheets were then soaked in thrombin solution for enhanced hemostasis. Material properties, such as density, tensile strength, biodegradability, and hemostatic capacity, were evaluated. Sheet efficacy was further assessed with liver bleeding and spinal venous plexus bleeding models in a miniature pig.

Results: High-density gelatin sheets showed stable shape retention in wet conditions and robust tensile strength. Sheets with higher density and more crosslinking had prolonged persistence in the pepsin test and lower biodegradability in vivo. Sheet B, produced from a 4% gelatin solution with heating at 155°C for 4 h, showed the best balance of properties, such as no deformation cracks, rapid water absorption, minimal expansion, and faster degradation within 10 weeks, compared with TachoSil and other sheets. In hemostasis models, Sheet B outperformed Avitene and TachoSil, achieving higher success rates in spinal (four out of six sites) and liver bleeding (five out of five sites) models.

Conclusions: A thrombin-loaded hemostatic sheet produced from 4% gelatin solution with a short heating time for crosslinking demonstrated well-balanced material properties, such as shape retention, biodegradability, and wet expansion rate, which resulted in effective hemostasis in in vivo models. These advances may contribute to surgical hemostatic applications.

Keywords:

Hemostasis, Gelatin, Thrombin, Biodegradability, Spinal Surgery

Spine Surg Relat Res 2025; 9(2): 218-225
dx.doi.org/10.22603/ssrr.2024-0147

Introduction

Despite surgical advances, intraoperative bleeding remains a significant issue in spinal surgery, requiring effective hemostasis for positive postoperative outcomes. Conventional methods such as compression, ligation, and thermocoagulation present risks like nerve damage and difficult-to-control bleeding in intricate surgical sites. Gauze, although commonly used, can obstruct the field and lead to rebleeding upon removal.

To overcome these challenges, gelatin or collagen-based hemostatic materials are used as alternatives[1,2)]. These materials, such as Gelfoam and Spongel, absorb blood and apply pressure to stop bleeding. However, in spinal surgery, they must be removed afterward to prevent nerve compression and require thin slicing for narrow sites.

Thrombin, a blood coagulant, is often administered with gelatin sponges for secondary hemostasis, but this process is

Corresponding author: Satoru Egawa, egawa.orth@tmd.ac.jp
Received: May 20, 2024, Accepted: August 10, 2024, Advance Publication: September 9, 2024

Table 1. Properties of the Hemostatic Sheets.

Gelatin Solution Concentration	Additional Heating Time at 155°C	Sheet Name	Sheet Density (mg/cm^3)	Thrombin-Loaded Sheet Density (mg/cm^3)	Shape Retention Angle (°)	Pepsin Disappearance Time (min)	Disappearance Rate In Vivo
4%	None	Sheet A	17.9±0.9 (n=20)	38.1±1.5 (n=20)	56±5 (n=10)	44	1/2 (50%)
4%	4 h	Sheet B	19.3±1.2 (n=20)	42.2±2.9 (n=20)	78±6 (n=10)	182	3/4 (75%)
4%	8 h	Sheet C	20.9±0.9 (n=20)	43.6±2.9 (n=20)	72±7 (n=5)	239	3/3 (100%)
4%	12 h	Sheet D	20.3±0.9 (n=20)	39.9±2.4 (n=20)	58±3 (n=5)	289	3/3 (100%)
6%	None	Sheet E	25.0±1.3 (n=20)	52.2±1.6 (n=20)	104±5 (n=5)	103	0/3 (0%)
6%	4 h	Sheet F	27.5±1.6 (n=20)	51.1±2.3 (n=20)	109±6 (n=5)	229	2/3 (67%)
6%	8 h	Sheet G	31.6±2.1 (n=20)	54.6±2.3 (n=20)	107±6 (n=5)	263	1/6 (17%)
6%	12 h	Sheet H	28.5±1.5 (n=20)	56.0±3.1 (n=20)	121±10 (n=5)	336	2/6 (33%)

complex and time-consuming. Dry gelatin sponges containing thrombin collapse during drying, hindering blood absorption, whereas fibrin sealants containing human fibrinogen and thrombin lack compressible materials. In particular, TachoSil, a collagen sheet infused with fibrinogen and thrombin, is not recommended for spinal surgery in Japan due to its thickness and rigidity.

The current landscape of hemostatic agents has significant limitations and challenges. The application of thrombin with gelatin sponges is intricate and time-consuming, while existing materials often collapse or lose their effectiveness during use. Products such as TachoSil are too thick and rigid for delicate spinal procedures, and many materials require removal post-surgery to avoid complications, adding further risk and complexity.

We have developed a novel hemostatic material designed to address these specific challenges. This new material is soft yet stiffens upon blood absorption, allowing it to conform to narrow surgical sites without becoming fragile. It incorporates a gelatin sponge with thrombin, is approximately 2 mm thick, and is designed to degrade in tissues without excessive expansion.

This study investigates the effects of various manufacturing processes on the material properties of this hemostatic agent and compares its efficacy with that of existing hemostatic materials. By addressing the limitations of current products, we aim to enhance the effectiveness and safety of hemostatic methods in spinal surgery, ultimately improving surgical outcomes.

Materials and Methods

Gelatin sponges loaded with thrombin from gelatin solution (Sheets A-H)

Bovine bone-derived gelatin (G3287P, Nitta Gelatin Inc., Osaka, Japan) was dissolved in purified water heated to 50°C to make gelatin solutions of 4% or 6%. The concentration of the gelatin solution in our novel material was determined through extensive trial and error, as there is no existing literature guiding the optimal concentration for spinal surgery applications. We settled on 4% and 6% gelatin solutions, which strike an ideal balance between mechanical strength and flexibility. The solution was mixed with air to create gelatin foam with densities of 0.33 g/mL or 0.29 g/mL using a 4% or 6% gelatin solution, respectively. The foams were dispensed into stainless steel or polyethylene containers and frozen at −40 to −30°C.

The frozen blocks were then half-thawed at 0°C and cut into appropriate sizes using a ham slicer (LH30, Hitachi Koki, Tokyo, Japan). They were placed in a freeze dryer (Lyoph-3, ULVAC, Kanagawa, Japan) at a shelf temperature of 0°C and dried under reduced pressure (13.3 Pa) for 96-141 h. The temperature was then increased to 60°C and the pressure was reduced to 0 Pa for further drying for 24-72 h to obtain a gelatin sponge. The sponge was then sliced to a thickness of approximately 3 mm using the ham slicer.

To obtain crosslinked gelatin sponge, the sliced sponge was placed in a dry heat sterilizer (DCH-120HL, Alp, Tokyo, Japan) and heated at 153°C for approximately 200 min, followed by further heating at 120°C for 7 h. The resulting crosslinked gelatin sponge was then subjected to an additional heat treatment at 155°C for 0 (no additional heat treatment), 4, 8, or 12 h. The resulting products were cut into sheets 50 mm long and 100 mm wide to obtain sheet carriers named Sheets A to H based on the process used (Table 1).

To load approximately 50 IU/cm^2 of thrombin, a solution of recombinant human thrombin (284 IU/mL) was prepared using a RECOTHROM 20,000 IU Topical Kit and RECOTHROM 5,000 IU Topical Kit from Baxter (Deerfield, Illinois, USA). Sheets A to H were soaked in 8.8 mL of thrombin solution until fully immersed. The soaked sheets were then frozen in a freeze dryer at −18°C, −8°C, and −10°C for 305 min, 600 min, and 125 min, respectively. They were dried at 10°C under reduced pressure (133.0 Pa) for 9-12 h, at 10°C under reduced pressure (73.0 Pa) for 10 h, and at 25°C under reduced pressure (0 Pa) for 2-6 h.

Gelatin sponges loaded with thrombin from commercially available sponges (Sheets Spo, H-Spo, and Gel)

Commercially available gelatin sponges, Spongel (Ethi-

con, Inc., Somerville, New Jersey, USA) and Gelfoam (Pfizer Inc., New York, New York, USA), were sliced to a thickness of 3 mm and immersed in recombinant human thrombin to obtain hemostatic sheets loaded with approximately 50 IU/cm^2 of thrombin. They were then referred to as Sheets Spo and Gel, respectively. The original Spongel was then heated at 155°C for 4 h, similar to the additional heat treatment described above. The sheet was sliced to a thickness of 2.6 mm and also immersed in recombinant human thrombin, resulting in Sheet H-Spo.

Dimensions (Sheets A-H)

Samples of the hemostatic sheets were cut into 10-mm squares. For each sample, the density, thickness, and weight were measured (n=20). The density was calculated by dividing the weight by the volume, where the volume was determined from the thickness and area of the squares.

Shape retention angle (Sheets A-H, Spo, H-Spo, and Gel)

The shape retention angle of the hemostatic sheets was assessed to determine their ability to maintain shape under wet conditions. Sheets were cut into 10 mm by 20 mm pieces and immersed in saline for 30 min. Subsequently, the sheets were placed on a steel rod, and images were taken over 25 s to measure the shape retention angle (n=3-10) (Fig. 1g).

Biodegradability in vitro (Sheets A-H)

The hemostatic sheet was cut into 50 mg pieces and placed in the Erlenmeyer flask containing 3100 U/mg pepsin solution (Wako Pure Chemical Industries, Osaka, Japan). The flask was shaken at a speed of 78 rpm in a thermostatic water bath (PERSONAL-11 from TAITEC, Aichi, Japan) set at a temperature of 37°C until no residual hemostatic sheet was observed. The disappearance time was measured (n=3).

Deformation tolerance (Sheets B and H)

Sheets B and H were specifically chosen for deformation tolerance tests due to their distinct material properties, representing a range of structural compositions within the hemostatic sheet series. Sheet B was selected for its standard composition, serving as a baseline for comparison. Sheet H, having a higher density, was selected to evaluate how increased density affects deformity tolerance. It was hypothesized that Sheet H might exhibit lower deformity tolerance than Sheet B due to its higher density. The deformation tolerance test evaluated the sheets' ability to withstand mechanical deformation without structural failure. Sheets B and H were pressed along the curved surface of a pipette tip (diameter: 10 mm) to simulate mechanical stress. The pressed sheets were then inspected under a microscope for the presence of cracks or tears, which would indicate failure.

Tensile strength (Sheets B, H-Spo, and Spo)

The tensile strength of the hemostatic sheets was assessed using a custom-built tensile testing apparatus. Sheets were cut into 15-mm squares and mounted on the apparatus with one side held by forceps and the other side secured with a double clip. A conical tube filled with water was attached to the clip, and water was gradually added to increase the load. The force required to break the sheet was measured using a force transducer, and the tensile strength was calculated from the maximum force applied before failure (n=3) (Fig. 1h).

Water absorption (Sheet B, Gelfoam, and TachoSil)

Sheet B, TachoSil, and Gelfoam were tested for water absorption by dropping 0.1 mL of phosphate buffer onto their surface. Time until liquid disappearance was recorded (n=4).

Expansion rate (Sheet B)

Sheet B samples were moistened with purified water and imaged over time. Expansion rate was calculated as the percentage change in width and thickness from pre-wetted measurements at various time points (n=4).

In vivo experiments

All animal experiments were performed after obtaining approval from the Animal Experiment Committee of our institution and were conducted according to the guidelines of our institution on the care and use of laboratory animals.

Spinal surgery bleeding model (Sheet B and Avitene)

A bleeding model for spinal surgery was created in an 11-month-old male miniature pig (NIBS). The miniature pig was chosen for this model due to the anatomical and physiological similarities between the pig and human spines, which include comparable vertebral size and structure. The pig was anesthetized with ketamine hydrochloride and maintained with a mixture of N_2O:O_2 and isoflurane. The lamina was removed to expose the dura matter, and an incision was made in the peripheral vein of the vertebral vein to induce bleeding. After confirming the bleeding, hemostasis sheets (Sheet B) and Avitene were applied near the bleeding site. The hemostasis process was observed, and additional sheets were applied if necessary. Hemostasis was considered complete when bleeding stopped for at least 30 s.

Liver bleeding model (Sheet B and TachoSil)

A liver injury model was established in a 21-month-old male miniature pig using the same anesthesia procedure as in the spinal surgery bleeding model. The liver was selected because it is a highly vascular organ, making it an excellent model for assessing the hemostatic properties of materials under conditions of significant bleeding. Pigs have a liver size and structure similar to humans, which facilitates the translation of experimental findings to potential clinical applications. A 12-mm-diameter damage plate was pressed against the liver surface, creating injury and bleeding. Hemostatic sheets (Sheet B) or TachoSil were applied to the injured area, and hemostasis was initiated. After application, bleeding was observed for 5 min. If there was no additional

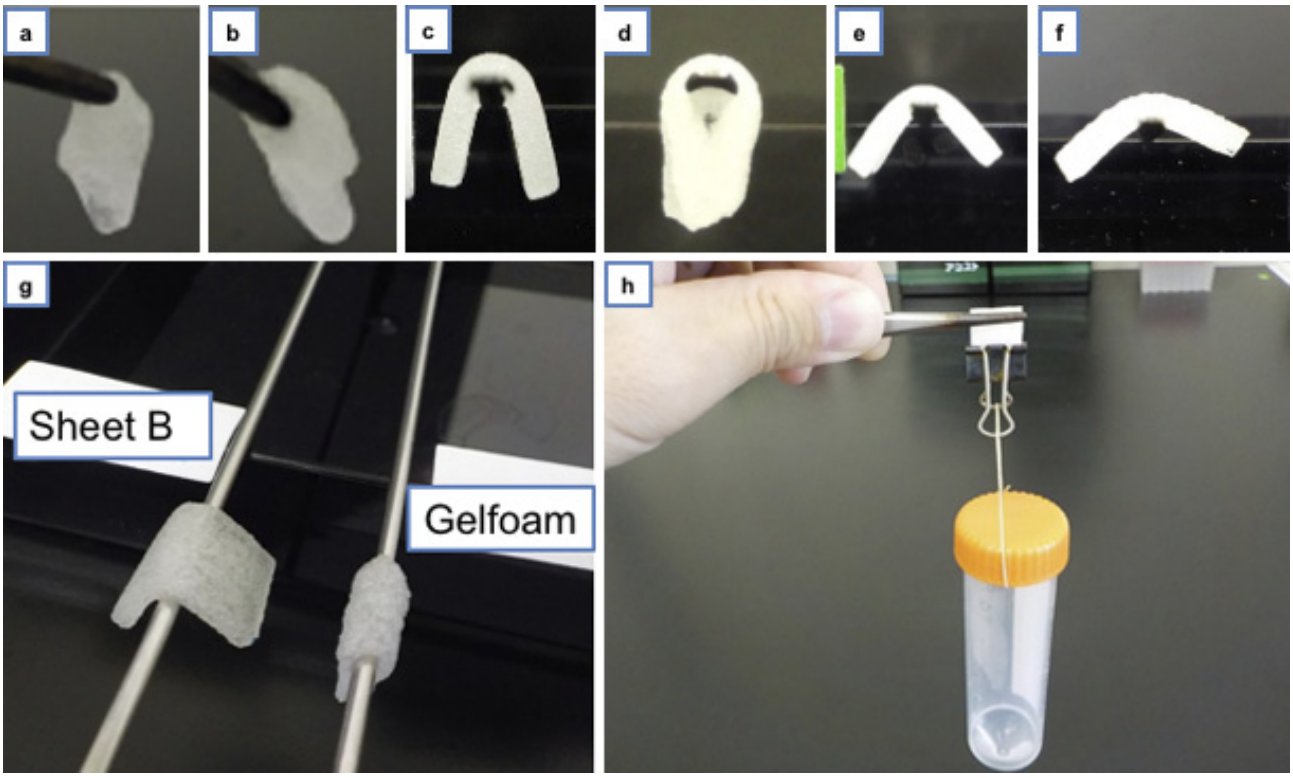

Figure 1. (a, b) Wetting states of Spongel and Gelfoam with a thrombin solution and measurement of shape retention angles. (c, d) Images of wet Sheets Spo and Gel, used to measure shape retention angles. (e, f) Images of wet Sheets B and F for measuring shape retention angles. (g) Photograph illustrating the method for testing wet shape retention ability. (h) Photograph showing the method for measuring tensile strength.

bleeding during this period, hemostasis was considered complete. If bleeding persisted after 6 min, the bleeding time was recorded.

Biodegradability in rats (Sheets A-H and TachoSil)

Rats (Wister strain, 7-15 weeks old, male) were anesthetized and laparotomized. Rats are commonly used in biodegradability studies due to their well-characterized physiology and ease of handling. An 8-mm-diameter hole was created on the liver surface, and Sheets A-H and TachoSil were applied to the injury site. After no rebleeding was confirmed, the laparotomy incision was closed and analgesia was administered. The disappearance of the hemostatic sheets was confirmed during a subsequent surgery several weeks later (n=2-6 rats).

Statistics

Statistical analysis was performed using IBM SPSS statistics for Macintosh, Version 25.0 (Released 2017 IBM Cor, Armonk, NY). t-tests were used to compare the averages of continuous variables, whereas chi-square tests were used to compare the proportions of categorical variables between the groups. A p-value of <0.05 was considered statistically significant.

Results

Dimensions (Sheets A-H)

Table 1 shows the manufacturing process, sheet density, and thrombin-loaded sheet density. Sheet density and thrombin-loaded sheet density were higher in the sheets made from 6% gelatin solution than in those made from 4% gelatin solution. Additional heat treatment largely did not affect the density.

Shape retention angle (Sheets A-L, Spo, H-Spo, and Gel)

Fig. 1 shows the appearance of the shape retention angle tests. In this Fig., Sheets B (left) and Gel (right) are placed side by side on metal rods, and Sheet B clearly tended to retain its original shape, which showed a larger retention angle Fig. 1(g). Fig. 1(a), 1(b) shows that Sheets Spo and Gel, which were made from commercially available gelatin sponges, became softened under wet conditions. Fig. 1(c), 1(d) present images of these wet Sheets Spo and Gel, which were used to measure their shape retention angles. On the other hand, the sheets that we manufactured from gelatin solution largely maintained a sheet shape and a wide angle even when wet, as shown in Fig. 1(e) (Sheet B), 1(f) (Sheet F). Table 1 describes all of the shape retention angles.

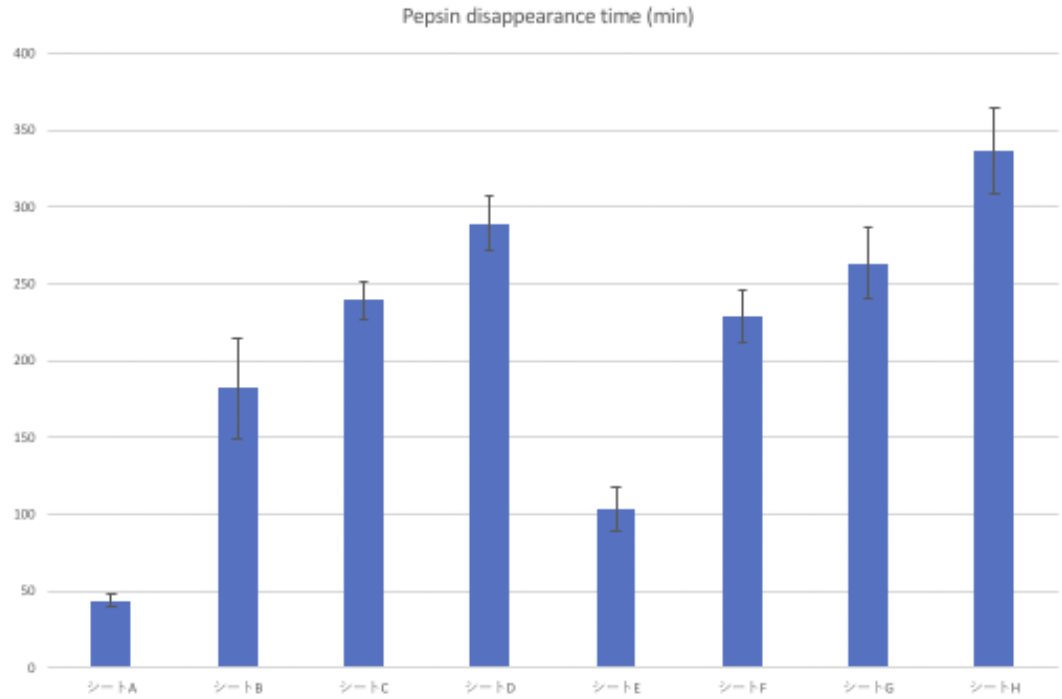

Figure 2. Graph showing the pepsin disappearance time of Sheets A–H. Notably, the sheets demonstrate increased resistance to biodegradation, a characteristic attributed to both the additional heat treatment and a higher concentration of the gelatin solution. Error bar showing standard deviation.

Table 2. Tensile Strength and Shape Retention Angle.

	Sheet SH	Sheet B	Sheet Spo	Sheet M
Tensile strength (g)	22±0.3	29±0.3	18±0.0	18±0.0
Shape retention angle (°)	64±5 (n=5)	78±6 (n=10)	20±3 (n=10)	8±2 (n=5)

Biodegradability in vitro (Sheets A-H)

The disappearance time with pepsin widely varied from 44 min (Sheet A) to 336 min (Sheet H) (Table 1, Fig. 2). This result indicates that both the additional heat treatment and the concentration of the gelatin solution make the sheet resistant to biodegradation.

Deformation tolerance (Sheets B and H)

No cracks and tears were observed in any of the five samples in Sheet B; however, cracks were observed in all five cases in Sheet H. An increase in density or excessive heat treatment might decrease deformation tolerance.

Tensile strength (Sheets B, Spo, and H-Spo)

Tensile strengths of Sheets B, Spo, and H-Spo were 29±0.3 g, 18±0.0 g, and 22±0.3 g, respectively (Table 2). This result indicated that heat treatment can increase the tensile strength of gelatin sponges and make them durable during hemostasis. In addition, the hemostatic sheets with a high shape retention angle when wet also had a high tensile strength. Moreover, heat treatment made the sponge durable (Fig. 3).

Water absorption (Sheet B, Gelfoam, and TachoSil)

The solution dropped on Sheet B was almost immediately absorbed, with the water absorption time being less than 1 s. In contrast, TachoSil (both sides) and Gelfoam created an interfacial tension at the contact surface between the sheet and the droplet, causing the droplet to be retained. Droplets were retained for more than 300 s. Our hemostatic sheet made of a crosslinked gelatin sponge sheet exhibited a higher water absorption capacity than existing hemostatic materials, indicating a higher hemostatic efficacy.

Expansion rate (Sheet B)

A width expansion of about 6% was observed in the wet condition, but no further expansion was detected (Table 3). A slight decrease in thickness was seen over time. The volume expanded by about 10%-11% at 1 h after wetting but returned to the same volume as before wetting after 3 h, according to calculations based on width and thickness changes.

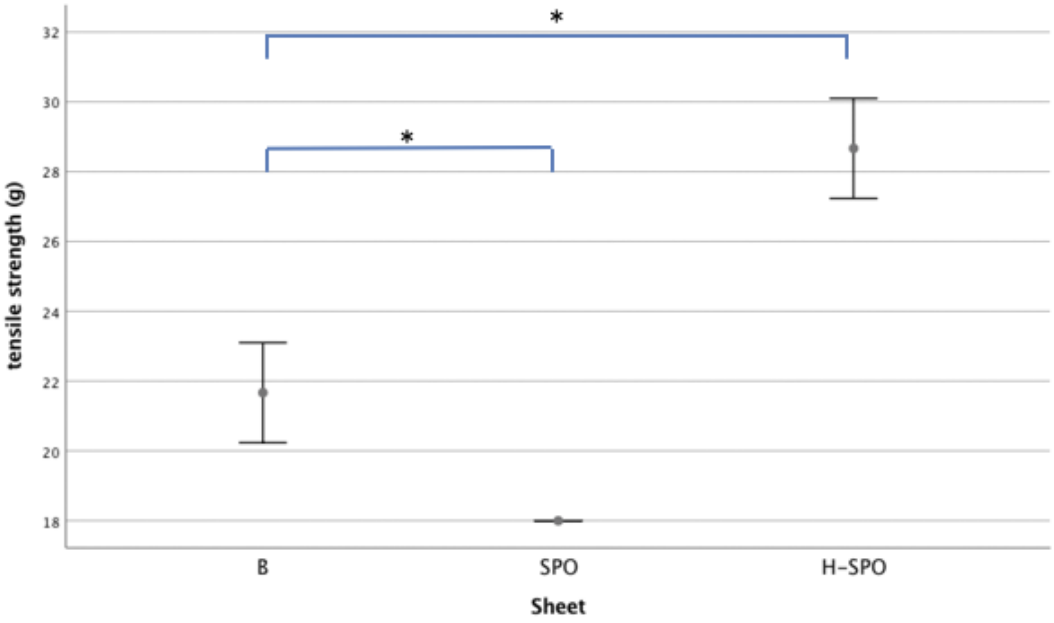

Figure 3. Graph showing the tensile strength of Sheets B, Spo, and H-Spo. Bars represent the 95% confidence interval, with the mean indicated by a dot. There were statistically significant differences between Sheets B and Spo and between Sheets B and H-Spo.

Table 3. Expansion Rate under Wet Conditions.

Time after wetting	Immediately	1 h	3 h	6 h
Width expansion (%)	5.9±5.4	5.9±6.1	3.9±5.5	2.8±3.6
Thickness expansion (%)	−2.4±8.0	−0.8±8.1	−6.6±2.3	−9.2±6.3

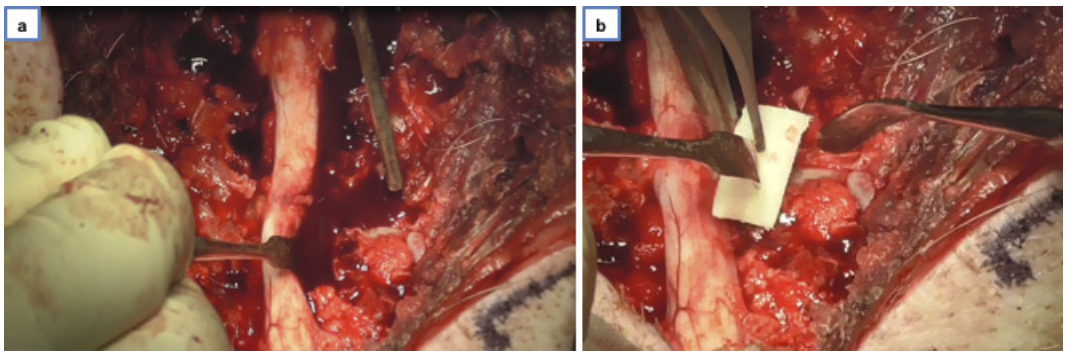

Figure 4. (a) Photograph of the bleeding model during pig spinal surgery, showing reproduced bleeding from the epidural vein. (b) Photograph demonstrating the application of hemostatic Sheet B to the bleeding point.

Hemostasis testing using a spinal surgery bleeding model with a miniature pig (Sheet B and Avitene)

Sheet B was applied to the left side and Avitene was applied to the right side at the same spinal level of the epidural venous plexus (Fig. 4(a), (b)). Hemostasis was achieved in four out of six bleeding sites with Sheet B and only one out of six bleeding sites in the Avitene group.

Hemostasis testing using a liver bleeding model with a miniature pig (Sheet B and TachoSil)

Hemorrhage from the liver surface was observed in three out of five cases with TachoSil, even 6 min after application. On the other hand, hemostasis was achieved in all injured sites (five out of five) with Sheet B and the hemostatic time was 1 min each.

Biodegradability in rats (Sheets A-H and TachoSil)

Most sheets of types A, B, C, and D disappeared within

10 weeks after implantation, whereas TachoSil and Sheets E, G, and H remained even 14 weeks after implantation, reflecting a lower disappearance rate (0%, 0%, 17%, and 33%, respectively) (Table 1).

Discussion

Topical hemostats can be classified into various categories, such as mechanical, bioactive, flowable, and sealant agents, with each serving distinct roles in managing bleeding[3]. Mechanical agents create physical barriers that slow bleeding and promote clot formation[4]. Bioactive hemostats enhance the clotting process through biological mechanisms[5]. Although active hemostats are particularly effective for patients with coagulation disorders, they tend to be costlier[6]. It is important to note that some animal or human-derived active hemostats have potential risks of immunogenicity and viral contamination, with pooled human plasma thrombin presenting a viral transmission risk[4]. In contrast, recombinant thrombin offers a lower risk of antibody formation compared with bovine thrombin[4,5]. The choice of materials for topical hemostats plays a crucial role in their effectiveness and safety[7-18].

Gelatin-based hemostatic sheets are considered a promising solution due to their biocompatibility and absorbable nature, making them well-tolerated by the body and simplifying postoperative care. To enhance the mechanical properties of gelatin, it can be heat-treated to promote crosslinking, thus increasing its strength and durability. In this study, we aimed to optimize the formulation and manufacturing process of these sheets by varying the concentration of the gelatin solution and applying different heat treatment parameters (supplementary file). Thrombin, known for its ability to enhance clotting, was incorporated into the sheets to augment their hemostatic capacity. The addition of thrombin is reported to enhance bleeding control. Floseal is a well-known flowable agent comprising crosslinked hydrolyzed bovine gelatin (500- to 600-μm particles) and human thrombin (500 IU/mL). It is a highly efficacious topical hemostatic agent but cannot apply physical pressure to bleeding points. Thus, it less effectively stops active bleeding than sheet-type hemostats. To overcome this, a dry gelatin sponge containing thrombin has been proposed. However, this type of sponge remains unavailable due to sponge collapse during drying, compromising its blood absorption capacity. In this study, we used an appropriate heat treatment and drying procedure to successfully develop a hemostatic sheet loaded with thrombin and with preserved blood absorption capacity. Upon application to a bleeding site, the gelatin-based sheet adheres to the tissue surface, forming a barrier that physically restricts blood flow and provides a scaffold for clot formation. The incorporation of thrombin into the sheets ensures localized activation of the clotting cascade, leading to rapid fibrin formation and clot stabilization. This dual-action mechanism—physical barrier formation and localized thrombin activity—effectively stops bleeding by enhancing clot formation and stability.

The resultant sheets underwent comprehensive testing to evaluate their mechanical properties, in vivo disappearance kinetics, and hemostatic efficacy. Sheet B, fabricated using a 4% gelatin solution and heat-treated at 155°C for 4 h, exhibited superior mechanical properties, such as excellent shape retention under wet conditions and robust tensile strength. Its selection as the optimal hemostatic agent was based on its moderate strength and favorable disappearance properties, which balanced mechanical performance and biodegradability, which are critical factors in effective topical hemostats.

In comparative studies using animal models, Sheet B demonstrated more effective hemostasis than Avitene in the pig spine venous plexus bleeding model and achieved hemostasis within 1 min in the pig liver bleeding model, outperforming TachoSil. This suggests that Sheet B has better hemostatic capacity not only for spinal surgery but also for general surgery.

The good disappearance property of Sheet B emphasizes its biodegradability, which is crucial for surgical settings in which the hemostatic agent may need to remain in contact with tissues for extended periods. However, sheets with high gelatin solution density tended to exhibit prolonged disappearance times, raising concerns about potential complications such as nerve compression and granulomatous reactions.

In summary, the relationship among gelatin concentration, heat treatment, and sheet density affects their mechanical properties. Although an increased gelatin concentration and heat treatment improve mechanical strength, they may hinder biodegradation. Consequently, Sheet B, subjected to heat treatment involving a 4% gelatin solution at 155°C for 4 h, emerged as the optimal choice, performing exceptionally well in an in vivo bleeding model.

However, this study has some limitations. First, this study was conducted using a miniature pig model, which, while valuable, may not fully replicate human physiology and the complexities of human spinal surgery. Therefore, the results may not be directly translatable to human patients. Second, the in vivo model primarily focuses on venous hemorrhage, and the effect on arterial hemorrhage remains unverified. Third, the in vivo biodegradability assessment was limited in duration. Long-term biodegradability and potential chronic inflammatory responses were not thoroughly evaluated. Further research is needed to address these limitations and explore the broader applications of Sheet B in various surgical scenarios.

In conclusion, our newly developed hemostatic sheet loaded with thrombin is effective for achieving hemostasis during surgery, particularly during spinal surgery.

Conflicts of Interest: The authors declare that there are no relevant conflicts of interest.

Sources of Funding: This research received no specific

grant from any funding agency in the public, commercial, or not-for-profit sectors.

Acknowledgement: We thank Keiichi Yoshihara, Yasuharu Kimura, Megumi Aoki, Hideki Ito, and Hikaru Saito (Astellas Pharma Inc.) for their advice and support.

Author Contributions: A.K. analyzed the data and wrote the original draft. T.Y. and S.E. conceived of, reviewed, and edited the paper. M.O. acquired the data. A.K., M.O., and T.Y. performed the investigation. T.Y. supervised the research. All authors contributed to the writing of the final paper. All authors approved the paper to be published and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethical Approval: All animal experiments were performed after obtaining approval from the Animal Experiment Committee of Tokyo Medical and Dental University (A2019-320A).

Informed Consent: Not applicable.

References

1. Jackson MR, Friedman SA, Carter AJ, et al. Hemostatic efficacy of a fibrin sealant-based topical agent in a femoral artery injury model: a randomized, blinded, placebo-controlled study. J Vasc Surg. 1997;26(2):274-80.
2. McKeage K. Raplixa™: a review in improving surgical haemostasis. Clin Drug Investig. 2015;35(8):519-24.
3. Spotnitz WD, Burks S. State-of-the-art review: hemostats, sealants, and adhesives II: update as well as how and when to use the components of the surgical toolbox. Clin Appl Thromb Hemost. 2010;16(5):497-514.
4. Vyas KS, Saha SP. Comparison of hemostatic agents used in vascular surgery. Expert Opin Biol Ther. 2013;13(12):1663-72.
5. Hickman DA, Pawlowski CL, Sekhon UDS, et al. Biomaterials and advanced technologies for hemostatic management of bleeding. Adv Mater. 2018;30(4):1700859.
6. Huang L, Liu GL, Kaye AD, et al. Advances in topical hemostatic agent therapies: a comprehensive update. Adv Ther. 2020;37(10):4132-48.
7. Schonauer C, Tessitore E, Barbagallo G, et al. The use of local agents: bone wax, gelatin, collagen, oxidized cellulose. Eur Spine J. 2004;13(Suppl 1):S89-96.
8. Chiara O, Cimbanassi S, Bellanova G, et al. A systematic review on the use of topical hemostats in trauma and emergency surgery. BMC Surg. 2018;18(1):68.
9. O'Shaughnessy BA, Schafernak KT, DiPatri AJ, et al. A granulomatous reaction to Avitene mimicking recurrence of a medulloblastoma. Case report. J Neurosurg. 2006;104(Suppl 1):33-6.
10. Seyednejad H, Imani M, Jamieson T, et al. Topical haemostatic agents. Br J Surg. 2008;95(10):1197-225.
11. Li D, Chen J, Wang X, et al. Recent advances on synthetic and polysaccharide adhesives for biological hemostatic applications. Front Bioeng Biotechnol. 2020;8:926.
12. Neveleff DJ. Optimizing hemostatic practices: matching the appropriate hemostat to the clinical situation. AORN J. 2012;96(5):S1-17.
13. Slezak P, Monforte X, Ferguson J, et al. Properties of collagen-based hemostatic patch compared to oxidized cellulose-based patch. J Mater Sci Mater Med. 2018;29(6):71.
14. Xu D, Ren Z, Chen X, et al. A randomized controlled trial on effects of different hemostatic sponges in posterior spinal fusion surgeries. BMC Surg. 2016;16(1):80.
15. Sabel M, Stummer W. The use of local agents: Surgicel and Surgifoam. Eur Spine J. 2004;13(Suppl 1):S97-101.
16. Menovsky T, Plazier M, Rasschaert R, et al. Massive swelling of Surgicel® Fibrillar™ hemostat after spinal surgery. Case report and a review of the literature. Minim Invasive Neurosurg. 2011;54(5-6):257-9.
17. Antisdel JL, West-Denning JL, Sindwani R. Effect of microporous polysaccharide hemospheres (MPH) on bleeding after endoscopic sinus surgery: randomized controlled study. Otolaryngol Head Neck Surg. 2009;141(3):353-7.
18. Tschan CA, Nie M, Schwandt E, et al. Safety and efficacy of microporous polysaccharide hemospheres in neurosurgery. Neurosurgery. 2011;69(Suppl 1):ons49-63.