

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-asianjournalsurgery.com

ORIGINAL ARTICLE

Two-layer sheet of gelatin: A new topical hemostatic agent

Toshitaka Takagi^{a,b}, Hiroyuki Tsujimoto^a, Hiroko Torii^{a,c},
Yuki Ozamoto^{a,c}, Akeo Hagiwara^{a,*}^a Department of Medical Life System, Doshisha University, Kyoto, Japan^b Department of Internal Medicine, Hushimi Okamoto Hospital, Kyoto, Japan^c Department of Surgery, Kusatsu General Hospital, Shiga, Japan

Received 2 June 2016; received in revised form 30 August 2016; accepted 7 September 2016

KEYWORDSanimal experiment;
gelatin;
TachoSil;
topical hemostatic
agent;
two-layer sheet

Summary *Background/objective:* Uncontrolled surgical bleeding is associated with increased morbidity, mortality, and hospital cost. Topical hemostatic agents available today have problems controlling hemostatic effects; furthermore, their handling is difficult and they are unsafe.

Methods: We devised a new hemostatic agent comprising gelatin sponge and film designed to be applied to the bleeding site, thereby creating a topical hemostatic agent made of gelatin alone. The gelatin was prepared by alkali treatment to eliminate viral activity. Hemostatic effects, surgical handling, and tissue reactions of the materials, namely a two-layer sheet of gelatin, TachoSil, and gelatin sponge, were evaluated using 21 dogs' spleens.

Results: The two-layer gelatin sheet and gelatin sponge exhibited superior hemostatic effects (100% hemostasis completed) compared with TachoSil (0–17% hemostasis). The gelatin matrix immediately absorbed blood flowing from wounds and activated the autologous components in the absorbed blood that promoted coagulation at the bleeding site. The two-layer gelatin sheet had the best surgical handling among the evaluated materials. Materials made of gelatin were associated with fewer inflammatory reactions compared with materials of TachoSil.

Conclusion: The two-layer sheet of gelatin is a useful topical agent because of its superior hemostatic effects and usability, and is associated with a lower risk of transmitting diseases and inflammatory reactions.

Copyright © 2016, Asian Surgical Association. Published by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Conflicts of interest: All contributing authors declare no conflicts of interest.

* Corresponding author. Department of Medical Life System, Doshisha University, 1-3 Tatara Miyakodani, Kyotanabe-shi, Kyoto-fu, Japan 610-0394.

E-mail address: marina.1108@zeus.eonet.ne.jp (A. Hagiwara).

<http://dx.doi.org/10.1016/j.asjsur.2016.09.007>

1015-9584/Copyright © 2016, Asian Surgical Association. Published by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Please cite this article in press as: Takagi T, et al., Two-layer sheet of gelatin: A new topical hemostatic agent, Asian Journal of Surgery (2016), <http://dx.doi.org/10.1016/j.asjsur.2016.09.007>

1. Introduction

To control bleeding during surgery, conventional procedures such as ligation, direct compression, electrocauterization, and clipping are used. Topical hemostatic agents are available for treating oozing blood or bleeding from regions difficult to access by conventional methods. Uncontrolled surgical bleeding is associated with increased morbidity and mortality, higher hospital costs, and postoperative adhesions and infections.^{1–3} Blood transfusions increase the risk of postoperative complications and have safety issues. Various hemostatic agents have been developed,^{3–7} but improvement is needed in terms of efficacy, ease of handling, and safety, especially during laparoscopic surgery.

Among hemostatic materials, TachoSil (CSL Behring, King of Prussia, PA, USA) is a ready-to-use agent that comprises an equine collagen matrix coated with human fibrinogen and human thrombin. TachoSil is widely used in many surgical specialties and has proven to be a valuable tool for several indications.^{6,8,9} TachoSil has shown clinical superiority in terms of hemostatic efficacy, duration of hospital stay, and postoperative utility for hepatic, cardiac, renal, lung, and pancreatic surgeries compared with conventional surgical procedures.^{10–14} However, TachoSil has problems with intraoperative handling, especially at sites that are difficult to access, and is associated with higher risks of viral infection, other transferable diseases, and allergic reactions resulting from human hemostatic components.²

To address the abovementioned problems, hemostatic agents that are safe and easy to handle and have sufficient hemostatic effects are needed. Gelatin-based hemostatic agents with or without fibrin components may be used during surgery^{15–17} However, the hemostatic effects of gelatin matrix are controversial compared with other topical hemostatic agents.⁶ Gelatin-based agents without fibrin or thrombin components may be able to solve the problems mentioned above when usability is improved. In the present study, gelatin almost completely eliminated immunogenicity and viral activity by alkali treatment.

The remainder of this article describes the utility of a newly developed topical hemostatic agent, two-layer sheet of gelatin. TachoSil and the new product were compared in splenic injuries in which hemostasis is not easily achieved.

2. Materials and methods

2.1. Preparation of hemostatic agents

2.1.1. Two-layer sheet of gelatin

Low endotoxin gelatin extracted from porcine skins (Type-I collagen, Medigelatin) with an isoelectric point of 5 was supplied by Nippi Co. Ltd. (Tokyo, Japan). The gelatin was dissolved in distilled water to concentrations of 1.0 wt% and 4.8 wt%. The gelatin 4.8 wt% solution was cast onto a polystyrene Petri dish (nontissue-culture treated; Corning Inc., Tokyo, Japan) and dried overnight on a clean bench at room temperature. The obtained film was slightly cross-linked by exposure to UV light for 2 minutes. The gelatin 1.0 wt% solution was cast onto a gelatin film on a Petri dish and placed in a deep freezer (MDF-U53V; SANYO Electric Co.,

Osaka, Japan) at -80°C for 30 minutes; it was then freeze dried for 24 hours in a vacuum freeze dryer (DRZ350WA; Advantec, Tokyo, Japan) in order to make a gelatin sponge. After freeze drying, a two-layered gelatin sheet composed of gelatin film and sponge layers 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 3 hours. The two-layer sheet of gelatin was cut into square sheets of 30 mm \times 30 mm immediately before use (Figure 1).

2.1.2. TachoSil

TachoSil, which is composed of collagen matrix, fibrinogen, and thrombin was used in accordance with the manufacturer's instructions. TachoSil was cut into square sheets measuring 30 mm \times 30 mm immediately before use.

2.1.3. Gelatin sponge

A gelatin sponge was prepared using the same methods outlined above as a two-layer sheet of gelatin without undergoing the process to create a gelatin film (i.e., only a matrix made of sponge layer was used). 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.) at 140°C for 3 hours. The gelatin sponge sheet was cut into square sheets measuring 30 mm \times 30 mm immediately before use.

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

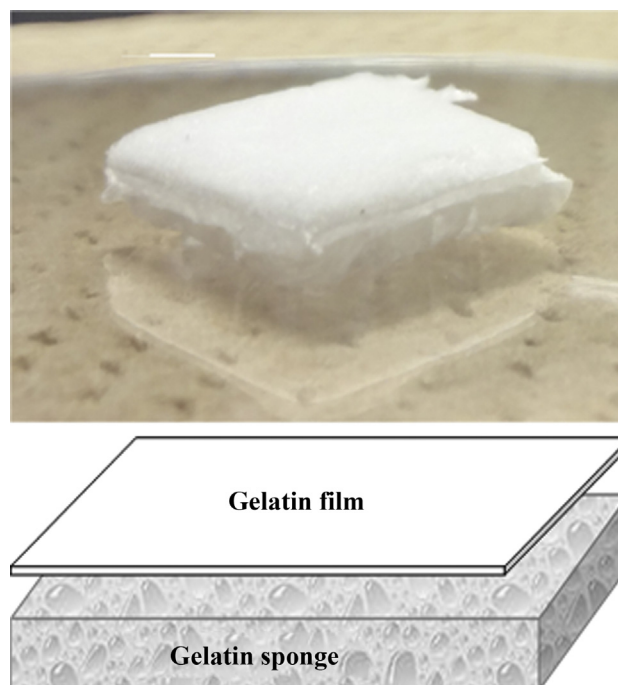


Figure 1 Two-layered gelatin sheet.

the animal care guidelines of the Committee for Animal Research of Doshisha University, Nara Medical University and European Commission Directive 86/609/EEC for animal experiments.

Twenty-one nonpregnant, female, 2-year-old beagles 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, mean temperature of 23°C, and mean humidity of 50%). Standard laboratory dog chow and water were freely available. Before the study, the dogs were housed in the laboratory for 1 week. On the 1st day of the experiment, the health condition of all dogs was assessed.

2.3. Surgical procedure to evaluate the effects of hemostatic agents

All surgeries were performed under sterile conditions, by a team of three persons. The dogs were randomly assigned to one of three groups corresponding to each hemostatic material. Eighteen dogs were anesthetized with intravenous sodium pentobarbital (Somnopentyl Kyoritsu Seiyaku, Tokyo, Japan; 34 mg/kg). A 12-cm epigastric median incision was made. The surface of the upper or lower part of the spleen was reduced with scissors to 1–2 mm in depth and 20 mm × 10 mm in area and allowed to bleed. Immediately after wiping blood from the spleen with gauze, one of three hemostatic materials (2-layer sheet of gelatin, TachoSil, gelatin sponge) was applied over the cut surface. Then, the hemostatic material was covered with gauze and digital pressure applied over it for 1 minute or 5 minutes; the gauze was removed gently so that immediate bleeding and rebleeding (bleeding after bleeding had stopped) could be observed during a 5-minute observation period. The physical status of the dogs remained stable during surgery. To exclude arbitrary procedure by operators or differences of conditions among experimental groups, the operators were blinded to the material being applied until astriction. Random study was scheduled for the experiments. This experiment was performed on six dogs for each hemostatic material. The time of rebleeding was recorded for each material.

Hemostatic effects were evaluated during an observation period of 5 minutes because the standard range in clinical tests using Duke's method is 1–5 minutes bleeding time. When blood was observed flowing out of the materials during the observation period, hemostasis was assessed as having been broken (rebleeding) and the time of rebleeding was recorded (Figure 2). The hemostatic materials were also evaluated for handling and ease of use during surgery.

2.4. Histological observation of implanted material and surrounding tissues

In order to observe histological change at the site of astriction, we examined three other dogs. The surfaces of the dogs' spleens were shaved to a size of 1 cm × 1 cm under general anesthesia, and bleeding was confirmed; astriction was performed for 1 minute with gauze. One of

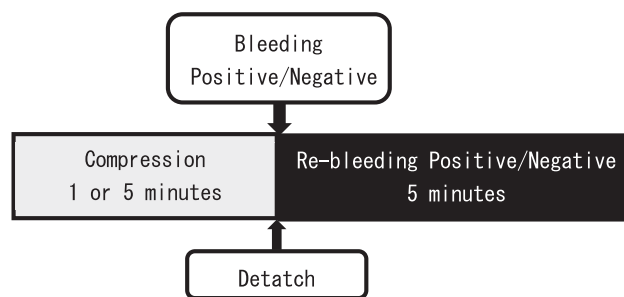


Figure 2 Schematic diagram of the experiment.

the three hemostatic agents was then applied to the wound and pressed for >5 minutes with gauze over the material. After hemostasis was confirmed, the abdomen was closed. The whole spleens were excised 2 weeks after the initial operation under inhalation anesthesia. The parts of the spleen that had been covered with each hemostatic material were resected, fixed with formalin, and stained with hematoxylin and eosin. The tissue response to the materials and residues of the hemostatic materials were observed.

2.5. In vitro experiments for absorbency and permeability

The absorbency and permeability of each hemostatic material was evaluated using canine blood. Hemostatic materials were cut into 1 cm × 1 cm squares. Single drops of 80 µL blood were placed by pipette on the hemostatic materials from a height of 1 cm. Absorption time was recorded as the time when the droplet disappeared from the material surface. Permeability was recorded as the time when the blood was exuded onto the material base.

2.6. Statistical analysis

The data are expressed as the mean ± standard deviation. Statistical analyses were carried out using the Kruskal–Wallis test and the chi-square test with Stat/Mate III, Windows (ATMS Co., Tokyo, Japan). A *p* value < 0.05 was considered statistically significant.

3. Results

3.1. Hemostasis effects in animal experiments

Table 1 shows the results for the three groups of hemostatic agents. In the TachoSil group, hemostasis was not achieved in three of six spleens after a minute's duration of compression of the bleeding site. In all the other spleens applied with TachoSil, rebleeding was observed during the 5-minute observation period after decompression. With respect to the other two groups, initial hemostasis was achieved after a minute of astriction and rebleeding was not seen in any of the six spleens during the observation period. There were significant differences between the TachoSil group and the two other groups (*p* < 0.001).

In all groups, hemostasis was successfully achieved after 5 minutes of compression. In five of six spleens in the

Table 1 Comparison of the hemostatic effects of TachoSil, a two-layer sheet of gelatin, and a gelatin sponge in bleeding dog spleens.

Hemostatic material	Positive for bleeding ^a / total experiments		Positive for rebleeding ^b / total experiments		Positive for bleeding ^{a+b} / total experiments	
	1 min	5 min	1 min	5 min	1 min	5 min
Two-layer sheet of gelatin	0/6	0/6	0/6	0/6*	0/6 *	0/6 *
TachoSil	3/6	0/6	3/6	5/6	6/6	5/6
Gelatin sponge	0/6	0/6	0/6	0/6*	0/6 *	0/6 *

* $p < 0.001$; significantly different compared with TachoSil.

^a Bleeding just after 1 minute or 5 minutes of compression.

^b Rebleeding occurred during the 5-minute observation period after 1 minute or 5 minutes of compression.

TachoSil group, rebleeding within 5 minutes was observed. In gelatin sheet and gelatin sponge groups, there was no rebleeding observed throughout the observation period (Table 1).

3.2. Absorbency and permeability of materials in vitro

The absorbency of the two-layer gelatin sheet (104 ± 115 seconds) and the gelatin sponge (277 ± 117 seconds) were significantly higher than that of TachoSil (>360 seconds; $p < 0.05$). Both the two-layer gelatin sheet and TachoSil had significantly ($p < 0.05$) low permeability (333 ± 61 seconds, > 360 seconds, respectively). The gelatin sponge had high permeability. That meant the two-layer sheet of gelatin had high absorbency and low permeability (Figure 3).

3.3. Usability of the materials during surgery

Blood from the spleen surface infiltrated the TachoSil and gelatin sponge and reached the compression gauze. The two kinds of gelatin preparations showed good adhesive properties because their sponge layer became an adherent gel after absorbing blood. By contrast, TachoSil was less adhesive after being soaked in blood and showed no gel

formation. The gel layer, composed of gelatin sponge and blood, attached tightly to the compression gauze, but it became difficult to remove the gauze from the material surface in the gelatin sponge group. However, the gelatin "film" layer was impermeable, and the blood from the wound did not reach the compression gauze. It was easy to detach the gauze without removing the gelatin sheet from the wound (Figure 4).

3.4. Histological findings regarding embedded materials

There was strong invasion of inflammatory lymphocytes noted in the injured part of the spleen embedded with TachoSil. The collagen materials of TachoSil still remained for 2 weeks. In the gelatin groups, inflammatory change was absent (Figure 5) and reepithelization was observed in 2 weeks. The gelatin materials were absorbed almost completely in 2 weeks. The wound healing in gelatin group was better than in TachoSil group.

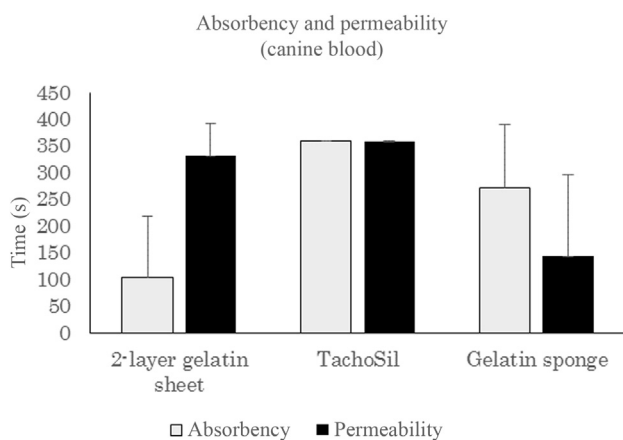
4. Discussion

Our experiments showed that the two-layer sheet of gelatin has superiority over TachoSil in terms of hemostatic effects, utility, and safety in canine experiments.

4.1. Hemostatic effect

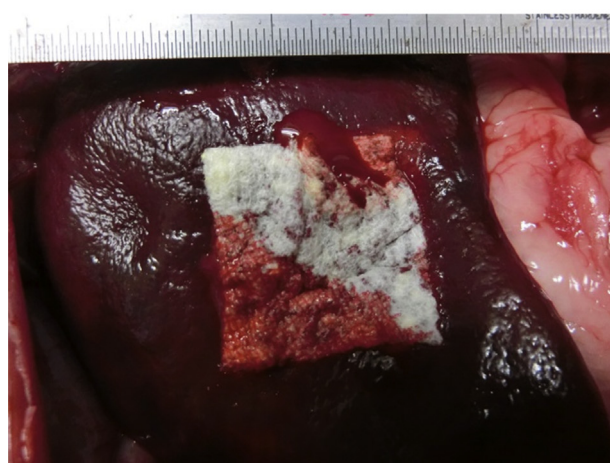
Topical hemostatic agents are used for the control of bleeding during surgery. Among the topical agents available, TachoSil has been shown to have superior hemostatic effects in various kinds of surgery.^{9,11–14,18} By contrast, TachoSil was less effective in sites where a relatively large quantity of bleeding was seen because of weak adhesion to the bleeding site.⁹ The hemostatic effect of gelatin matrix is still controversial,^{3,7,15,19,20} but most of the studies we found showed that gelatin had a poor hemostatic effect compared with TachoSil. This prompted us to develop a two-layer sheet of gelatin to solve these problems.

The physical properties of gelatin matrix contribute to superior hemostatic effects compared with collagen matrix (TachoSil). In our study, the gelatin sponge layer smoothly and rapidly absorbed blood and activated autologous blood-

**Figure 3** Absorbency and permeability of the materials.



a



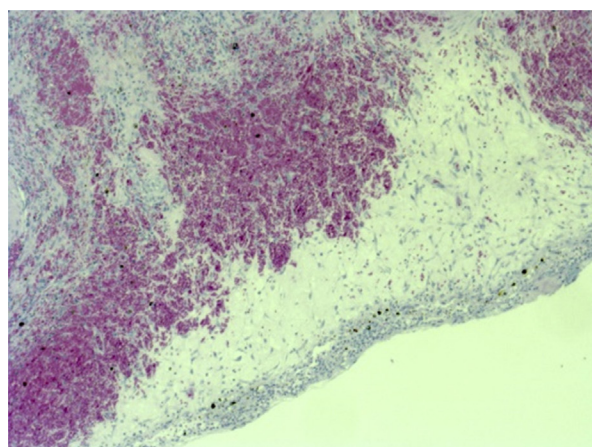
b



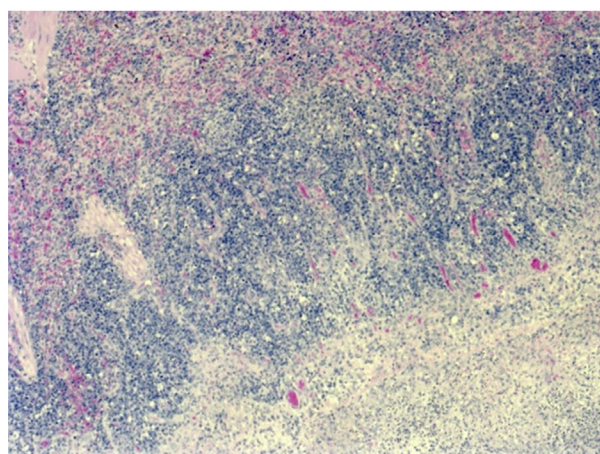
c

Figure 4 Hemostatic agents set onto the shaved surface of the spleen. (A) Two-layer sheet of gelatin. (B) TachoSil. (C) Gelatin sponge.

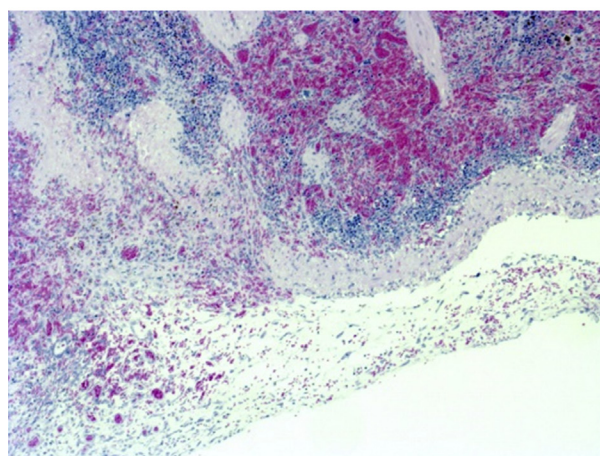
coagulating components in blood (Figure 2). The sponge matrix changed to gel, which covered bleeding sites tightly. Moreover, the gelatin film layer of the two-layer gelatin sheet inhibited the permeation of blood, consequently



a



b



c

Figure 5 Histologic findings regarding embedded materials. (A) Two-layer sheet of gelatin. (B) TachoSil. (C) Gelatin sponge.

strengthening adhesive bonding to the bleeding site. By contrast, the reduced permeability of the collagen sponge probably permitted continuous bleeding from the site, and subsequent bleeding or oozing disturbed the hemostatic effects of fibrinogen and thrombin because of the blood flowing out. The two-layer sheet of gelatin absorbed blood

flowing into the material and coagulation occurred quickly, thereby allowing fibrin to bind the sheet and wound tightly. After hemostasis was completed, the hemostatic effects were not necessary. The thickness of the two-layer sheet of gelatin was thought to be enough to stop bleeding in this study. However, TachoSil absorbed flowing blood more slowly and coagulation inside TachoSil and on the surface of the material took longer to complete. Regarding the rebleeding, flexibility and adherence of the materials were thought to be more important than absorbency. The two-layer sheet of gelatin's ability to follow the shape of the wound surface is superior to that of TachoSil. The gelatin sheet can adhere to the tissue tightly after compression, but TachoSil leaves a small gap after astringency. Rebleeding can occur from a gap between the material and the wound.

In our study, we set the time for manual compression at 1 minute or 5 minutes to compare the different hemostatic materials. In past studies, compression time was set at longer than 3 minutes. Compared with the other materials, the initial hemostasis of TachoSil took longer and was incomplete, resulting in more frequent instances of rebleeding. The two-layer sheet of gelatin required a shorter time to complete hemostasis and the hemostatic effects were so secure that no rebleeding was found in any of the spleens tested. Overall, the hemostatic effect of the two-layer sheet of gelatin appears to be superior to TachoSil in sites where a relatively large quantity of bleeding is seen (e.g., spleen, pelvic floor, blood flow-rich parenchymal organs).^{7,21,22} In the present study, we evaluated the hemostatic effects of three materials on a bleeding parenchymatous organ where bleeding is notoriously difficult to control. Conventional techniques for hemostasis and TachoSil are effective in cases where there is a small amount of bleeding and in sites where bleeding is easy to control. A two-layer sheet of gelatin is a possible choice for hemostasis in cases where there is a relatively large amount of bleeding.

4.2. Handling

The nonadhesive property of a gelatin "film" layer works more effectively to prevent bleeding immediately after the release of gauze compression. TachoSil and the gelatin sponge have a permeable layer and thus are disadvantageous for surgical handling because strong adhesion to compression gauze is more likely with the contact of blood and gauze, which may induce rebleeding after detachment. TachoSil is probably effective in cases where blood does not reach the compression gauze. The two-layer gelatin sheet and TachoSil present problems for laparoscopic surgery because they are both difficult to place into the abdominal cavity through a port. Gelatin materials must undergo refinements in order to be suitable for laparoscopic surgery. We are reforming gelatin materials for use during laparoscopic surgery.

4.3. Safety

Hemostatic agents remain in the body after surgery, therefore, long term safety is a very important issue. Excessive inflammatory response of the tissue by

hemostatic materials can cause adhesion. Moreover, long term residues of the material possibly make the risk of infection higher. In the present study histological examination was carried out in order to observe the tissue response to the each material, not to examine the hemostatic effects of each material. Earlier regeneration of the peritoneum and earlier absorption of the material were observed in two-layer gelatin sheet. However, TachoSil induced intensive inflammatory response at the implanted site and remained longer than the gelatin sheet. Regarding adhesion, two-layer sheet of gelatin is possibly safer than TachoSil.^{23–25} In our unpublished study (under submission) the two-layer gelatin sheet has an antiadhesive effect due to early regeneration of the peritoneum and mild inflammatory response.

The two-layer gelatin sheet, which is composed of alkaline-treated gelatin, does not carry the same risks as biomaterials (TachoSil), such as transmission of viral infection and allergic reactions. In fact, the gelatin alkali-treatment process eliminates the risk of transmissible diseases almost completely.²⁶ Moreover, from the skin of animals, infectivity of Bovine Spongiform Encephalopathy was not detected.

5. Conclusion

We showed that the two-layer gelatin sheet is a more effective, easier to handle, and safer topical hemostatic agent than TachoSil, which is one of the most popular materials currently in use. The two-layer sheet of gelatin is safer than topical agents including fibrin components and/or thrombin in terms of risk of viral transmission and inflammatory reactions. We showed the hemostatic effectiveness of a two-layer sheet of gelatin in bleeding dog spleens (an organ where bleeding is difficult to control). The efficacy of newly developed materials should be evaluated by application to other organs (liver, kidney, lung, and vessels) and in humans.

Acknowledgments

This work was supported, in part, by a grant from the Science and Engineering Institute of Doshisha University.

References

- Shander A, Kaplan LJ, Harris MT, et al. Topical hemostatic therapy in surgery: bridging the knowledge and practice gap. *J Am Coll Surg*. 2014;219:570–579. <http://dx.doi.org/10.1016/j.jamcollsurg.2014.03.061>. e4.
- Gruen RL, Brohi K, Schreiber M, et al. Haemorrhage control in severely injured patients. *Lancet*. 2012;380:1099–1108. [http://dx.doi.org/10.1016/S0140-6736\(12\)61224-0](http://dx.doi.org/10.1016/S0140-6736(12)61224-0).
- Neveleff DJ. Optimizing hemostatic practices: matching the appropriate hemostat to the clinical situation. *AORN J*. 2012; 96:S1–S17. <http://dx.doi.org/10.1016/j.aorn.2012.08.005>.
- Emilia M, Luca S, Francesca B, et al. Topical hemostatic agents in surgical practice. *Transfus Apher Sci*. 2011;45:305–311. <http://dx.doi.org/10.1016/j.transci.2011.10.013>.
- Sanders L, Nagatomi J. Clinical applications of surgical adhesives and sealants. *Crit Rev Biomed Eng*. 2014;42:271–292.

6. Kakaei F, Seyyed Sadeghi MS, Sanei B, et al. A randomized clinical trial comparing the effect of different haemostatic agents for haemostasis of the liver after hepatic resection. *HPB Surg.* 2013;2013. <http://dx.doi.org/10.1155/2013/587608>. Article ID 587608, 5 pages.
7. Wagner WR, Pachence JM, Ristich J, Johnson PC. Comparative *in vitro* analysis of topical hemostatic agents. *J Surg Res.* 1996; 66:100–108. <http://dx.doi.org/10.1006/jsre.1996.0379>.
8. Colombo GL, Bettoni D, Di Matteo S, et al. Economic and outcomes consequences of TachoSil: a systematic review. *Vasc Health Risk Manag.* 2014;10:569–575. <http://dx.doi.org/10.2147/VHRM.S63199>.
9. Maisano F, Kjærgård HK, Bauernschmitt R, et al. TachoSil surgical patch versus conventional haemostatic fleece material for control of bleeding in cardiovascular surgery: a randomised controlled trial. *Eur J Cardio-Thoracic Surg.* 2009;36:708–714. <http://dx.doi.org/10.1016/j.ejcts.2009.04.057>.
10. Kauvar DS, Lefering R, Wade CE. Impact of hemorrhage on trauma outcome: an overview of epidemiology, clinical presentations, and therapeutic considerations. *J Trauma.* 2006; 60:S3–S11. <http://dx.doi.org/10.1097/01.ta.0000199961.02677.19>.
11. Lang G, Csekeö A, Stamatis G, et al. Efficacy and safety of topical application of human fibrinogen/thrombin-coated collagen patch (TachoComb) for treatment of air leakage after standard lobectomy. *Eur J Cardiothorac Surg.* 2004;25: 160–166.
12. Anegg U, Lindenmann J, Matzi V, Smolle J, Maier A, Smolle-Jüttner F. Efficiency of fleece-bound sealing (TachoSil) of air leaks in lung surgery: a prospective randomised trial. *Eur J Cardiothorac Surg.* 2007;31:198–202. <http://dx.doi.org/10.1016/j.ejcts.2006.11.033>.
13. Siemer S, Lahme S, Altziebler S, et al. Efficacy and safety of TachoSil as haemostatic treatment versus standard suturing in kidney tumour resection: a randomised prospective study. *Eur Urol.* 2007;52:1156–1163. <http://dx.doi.org/10.1016/j.eururo.2007.04.027>.
14. Frilling A, Stavrou GA, Mischinger HJ, et al. Effectiveness of a new carrier-bound fibrin sealant versus argon beamer as haemostatic agent during liver resection: a randomised prospective trial. *Langenbecks Arch Surg.* 2005;390:114–120. <http://dx.doi.org/10.1007/s00423-005-0543-x>.
15. Schuhmacher C, Pratschke J, Weiss S, et al. Safety and effectiveness of a synthetic hemostatic patch for intraoperative soft tissue bleeding. *Med Devices (Auckl).* 2015;8:167–174. <http://dx.doi.org/10.2147/MDER.S79556>.
16. Dimitroulis D, Antoniou E, Karidis NP, Kontzoglou K, Kouraklis G. Surgical control of life-threatening postERCP bleeding with a gelatin matrix-thrombin hemostatic agent. *Int J Surg Case Rep.* 2012;3:471–473. <http://dx.doi.org/10.1016/j.ijscr.2012.05.014>.
17. Shukla A, Fang JC, Puranam S, Jensen FR, Hammond PT. Hemostatic multilayer coatings. *Adv Mater.* 2012;24:492–496. <http://dx.doi.org/10.1002/adma.201103794>.
18. Simo KA, Hanna EM, Imagawa DK, Iannitti D. Hemostatic agents in hepatobiliary and pancreas surgery: a review of the literature and critical evaluation of a novel carrier-bound fibrin sealant (TachoSil). *ISRN Surg.* 2012;2012:1–12. <http://dx.doi.org/10.5402/2012/729086>.
19. Chalupová M, Suchý P, Pražanová G, Bartošová L, Sopuch T, Havelka P. Local tissue reaction after the application of topical hemostatic agents in a rat partial nephrectomy model. *J Biomed Mater Res Part A.* 2012;100A:1582–1590. <http://dx.doi.org/10.1002/jbm.a.34098>.
20. Charlesworth TM, Agthe P, Moores A, Anderson DM. The use of haemostatic gelatin sponges in veterinary surgery. *J Small Anim Pract.* 2012;53:51–56. <http://dx.doi.org/10.1111/j.1748-5827.2011.01162.x>.
21. Larkin JO, Carroll PA, McCormick PH, Mehigan BJ. Control of splenic bleeding during splenic flexure mobilisation by devascularisation of the inferior pole of the spleen. *Tech Coloproctol.* 2012;16:459–461. <http://dx.doi.org/10.1007/s10151-012-0840-7>.
22. Matonick JP, Hammond J. Hemostatic efficacy of EVARREST, fibrin sealant patch versus TachoSil in a heparinized swine spleen incision model. *J Invest Surg.* 2014;27:360–365. <http://dx.doi.org/10.3109/08941939.2014.941444>.
23. Hu Y, Yamashita K, Tabayashi N, et al. Gelatin sealing sheet for arterial hemostasis and antiadhesion in vascular surgery: a dog model study. *Biomed Mater Eng.* 2015;25:157–168. <http://dx.doi.org/10.3233/BME-151266>.
24. Kang BS, Na YC, Jin YW. Comparison of the wound healing effect of cellulose and gelatin: an *in vivo* study. *Arch Plast Surg.* 2012; 39:317–321. <http://dx.doi.org/10.5999/aps.2012.39.4.317>.
25. Eren E, Başoğlu MS, Kulduk E, Şimsek F, İnan S. Mucosal trauma induced apoptosis in guinea pig middle ear: comparison of hemostatic agents. *Int J Pediatr Otorhinolaryngol.* 2014;78: 2222–2228. <http://dx.doi.org/10.1016/j.ijporl.2014.10.017>.
26. Grobber AH, Steele PJ, Somerville RA, Taylor DM. Inactivation of the bovine-spongiform-encephalopathy (BSE) agent by the acid and alkaline processes used in the manufacture of bone gelatine. *Biotechnol Appl Biochem.* 2004;39:329–338. <http://dx.doi.org/10.1042/BA20030149>.