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ORIGINAL ARTICLE

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

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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.

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

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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 × 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 × 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 × 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

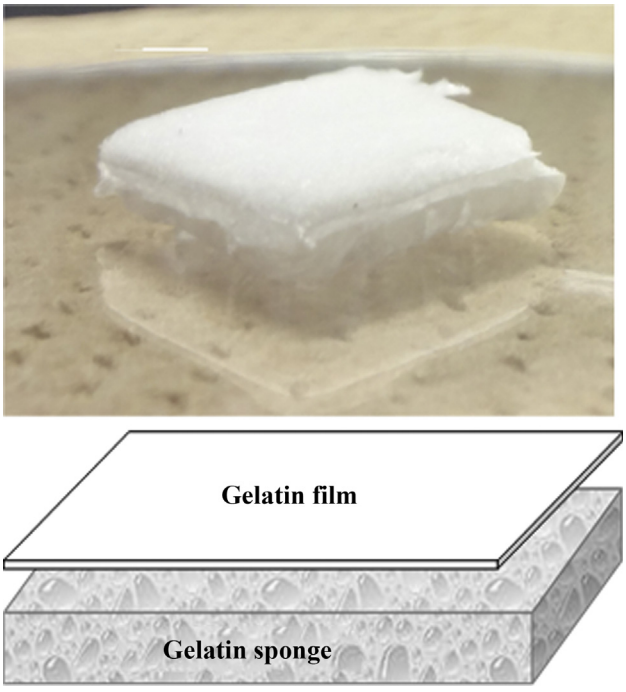

Figure 1 Two-layered gelatin sheet.

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