Effect of Thrombin Concentration on the Adhesion Strength and Clinical Application of Fibrin Glue-Soaked Sponge

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Abstract

Fibrin glue-soaked gelatin sponge (FGGS) has been used for tissue sealing in neurosurgical practice, but too rapid clotting of fibrin glue occasionally prevents good fixation of FGGS. Dilution of thrombin may provide adequate manipulation time between mixing fibrinogen and thrombin on gelatin sponge and application into the tissue defects. The present study characterized the effect of thrombin dilution on the adhesion strength of FGGS and retrospectively assessed the clinical usage of the dilution for filling dead space or sealing arachnoid defect in 255 cases who underwent transsphenoidal surgery for the last 66 months. FGGS was prepared using three different concentrations of thrombin: 250 (standard), 50 (1:5 dilution), and 25 (1:10 dilution) units/ml, and incubated for three different periods (5, 20, and 60 seconds). FGGSs were applied over two adjacently positioned porcine skins placed on two metallic plates. The adhesion strength was evaluated by measuring maximum tensile strength during pulling out the sliding plate at a constant rate of displacement. The maximum adhesion strength was greater for FGGS with 1:10 diluted thrombin solution than for FGGS prepared with higher concentrations ($p < 0.05$). Adhesion strength did not decay for 20 seconds after the mixture. Only four of 255 cases (1.6%) required second reconstruction of sella floor due to the cerebrospinal fluid leakage. FGGS prepared with diluted thrombin solution can provide adequate adhesion strength for clinical use.

Key words: fibrin glue-soaked sponge, skull base, adhesion strength, transsphenoidal approach, reconstruction

Introduction

Cerebrospinal fluid (CSF) leaks are considered serious complications in neurosurgical procedures and efficient tissue sealing is an important preventative maneuver. Fibrin glue has been commercially available for more than 20 years and currently is being used in neurosurgical practice in many countries as a fixative of injured tissue, for hemostasis, and for the prevention of CSF leakage.^{1,4,8,14,16,18,19,21} The usefulness of collagen sponge, singly or in combination with fibrin sealants or artificial materials, has also been reported for dural repair. Collagen sponge with fibrin glue has yielded good results in the prevention of CSF leakage.^{9,11,13,14} We have used fibrin glue-soaked gelatin sponge (FGGS) for tissue sealing in neurosurgical practice, especially for transsphenoidal surgery, for more than 10 years.

However, too rapid clotting of fibrin glue occasionally prevents good fixation of the FGGS to the surrounding tissues. On such occasions, we have repeated the procedure using FGGS with diluted thrombin solution, resulting in good fixation. Based on these experiences, we have primarily employed this dilution technique to prepare FGGS for sellar reconstruction since April 2006, with good potential to prevent postoperative CSF leakage.

To confirm the adequacy of our clinical procedures, we performed experiments to evaluate the effect of thrombin dilution on the adhesion strength of FGGS, and retrospective assessment of sellar reconstruction using FGGS prepared with diluted thrombin solution (FGGS-DT).

Materials and Methods

Bolheal® fibrinogen (80 mg/ml + blood coagulation factor XIII 75 units/ml + bovine aprotinin 1000 units/ml) and thrombin solution (250 units/ml + cal-

Fig. 1 Schematic illustration of the device used for measuring adhesion strength of fibrin glue-soaked gelatin sponge (FGGS) to the porcine skin graft. Two porcine skin grafts were placed adjacent, one on a fixed plate and the other on the sliding plate which was hooked to the traction speed measurer. Skin grafts were clipped on the slides. FGGS was then placed onto the skin graft junction and a sinker (123 g) was placed for compression for 3 minutes. Traction was applied at 50 mm/min by a speed controller. Then the drawing strength (J) was evaluated by a strength measurer attached to the speed controller.

cium chloride 40 $\mu\text{mol/ml}$) were obtained from The Chemo-Sero-Therapeutic Research Institute, Kumamoto, Kumamoto. Absorbable gelatin compressed sponge (Gelfoam®) was obtained from Pfizer, New York, New York, USA. One hundred eight pre-treated porcine skin grafts were stored at -18°C after removal of fat to avoid variations in the adhesion strength. Just before the experiment, the skin grafts were unfrozen at room temperature, moisturized, and then wiped with gauze. Porcine skin grafts and measuring devices were prepared and operated according to standard test method for wound closure strength of tissue adhesives and sealants, F2458, 2005.²⁾

Two porcine skin grafts were placed on metallic jig plates (IDM Corporation, Koshi, Kumamoto) adjacent to one another and fixed on the plates with clips (Fig. 1). Three solutions with different concentrations of thrombin were prepared: 250 units/ml (standard), 50 units/ml (1:5 dilution, prepared by mixing 200 μl of standard thrombin solution with 800 μl of 0.9% physiological saline), and 25 units/ml (1:10 dilution, prepared by mixing 100 μl of standard thrombin solution with 900 μl of 0.9% physiological saline). The surface of a gelatin sponge piece was briefly dipped in fibrinogen solution and then dipped in one of the three thrombin solutions. For each thrombin concentration, the FGGS was incubated for 5, 20, or 60 seconds. The FGGS was then placed onto the skin graft junction and a sinker (123 g) was placed over the FGGS, to apply compression for 3 minutes. Traction was applied at 50 mm/min using a speed controller (Model-2257; Aikoh En-

Fig. 2 Operating microscope views of the clinical application of fibrin glue-soaked gelatin sponge prepared with diluted thrombin solution (FGGS-DT) in a case of prolactinoma. **A:** Cerebrospinal fluid leakage was seen from the arachnoidal tear behind the tuberculum sellae (arrows) at the end of tumor removal. **B:** FGGS-DT was applied to the leakage point to primarily seal the defect. **C:** After packing of intrasellar dead space with the FGGS-DT, the sellar floor was reconstructed with a vomer splint. **D:** Reconstructed sellar floor was covered by FGGS-DT.

gineering Co., Ltd., Higashiosaka, Osaka), and the drawing strength in joules (J) was evaluated by a strength measurer (Model-9500; Aikoh Engineering Co., Ltd.) attached to the controller. The maximum drawing strength, always measured just before peeling of the FGGS away from the porcine skin graft, was considered to be the adhesion strength of FGGS. This procedure was repeated six times for each concentration and for each incubation time. Data on adhesion strength were collected, and means and standard deviation were calculated. Statistical differences between the adhesion strength, of different groups were determined by Tukey's multiple comparison tests at the 95% confidence level.

FGGS-DT was used for reconstruction of the sellar floor in 255 patients who underwent transphenoidal surgery for pituitary lesions from April 2006 to September 2011. The lesions included 195 pituitary adenomas, 22 Rathke's cleft cysts, 10 craniopharyngiomas, 8 chordomas, 3 meningiomas, 3 germinomas, and 14 miscellaneous pathologies. Intraoperative CSF leakage was observed in 27 cases. After aspirating the CSF around the defect of the arachnoid membrane, the leakage point was primarily sealed with FGGS-DT (1:5 or 1:10) (Fig. 2).

Then the dead space in the sella turcica was also filled with FGGS-DT. The sellar floor was reconstructed with a vomer splint. Two to three pieces of FGGS-DT were applied to cover the sellar floor. For wide defects of arachnoid membrane, not uncommon in transsphenoidal surgery of craniopharyngioma or meningioma, two to three pieces of FGGS-DT were applied for primary sealing of the defects, then adipose tissue was inserted under the FGGS-DT layer to secure the fixation. The sellar floor was reconstructed in the same manner. Spinal drainage of CSF was continued for 3 days in these cases. In cases without intraoperative CSF leakage, the dead space was filled with FGGS-DT, with the same procedure.

Results

The mean adhesion strength of the FGGS-DT prepared with 1:10 diluted thrombin solution was greater than those of FGGS-DT prepared with other solutions for all incubation times (Table 1). Analysis of variance and post-hoc Tukey's multiple comparison tests revealed no significant differences in the adhesion strengths between the three different incubation time groups when the standard thrombin was used. Maximum adhesion strength was observed for FGGS-DT was incubated for 5 seconds using 1:5 and 1:10 dilutions, but the difference from those incubated for 20 seconds was not statistically significant.

FGGS-DT was applied in 255 cases for the reconstruction of sellar floor with/without sealing of tear of arachnoid membrane at the end of transsphenoidal surgery. Autologous adipose tissue was also used in 19 cases. Postoperative CSF rhinorrhea needing second reconstruction of sellar floor ensued in only 4 cases (1.6%) including a case of craniopharyngioma which needed extensive subarachnoid manipulation. No autologous adverse effects of FGGS were encountered.

Table 1 Adhesion strength of fibrin glue-soaked gelatin sponge to porcine skin graft

Dilution	Incubation time (sec)		
	5	20	60
1:1	0.54 ± 0.15	0.55 ± 0.20	0.47 ± 0.10
1:5	1.17 ± 0.30*	0.96 ± 0.21*	0.66 ± 0.12**
1:10	1.34 ± 0.13*	1.19 ± 0.23*	0.95 ± 0.27*,**

Values are mean ± standard deviation in J. *p < 0.05 compared with 1:1 dilution in the same incubation time group; **p < 0.05 compared with 5 sec using the same dilution.

Discussion

Our experimental study showed that 1:10 dilution of commercially available thrombin solution provides the greatest adhesion strength for FGGS-DT compared with higher concentration solutions. We posit that slow conjugation time with thinner thrombin gives enough time for the fibrinogen-thrombin mixture to spread onto the irregular surface of tissue before hardening, which provides stronger attachment of FGGS to the tissue. In addition, studies in tissue engineering have reported that increased thrombin concentration modifies the microfibril structure of the fibrin gels, by decreasing the fiber bundling size and so decreasing the porosity of the gel, which may lead to reduced adhesion strength.^{3,12,15)} Maximum adhesion strength of autologous fibrin glue was reported with thrombin concentration of 50 units/ml, and a significant decrease with thrombin concentrations over 200 units/ml, suggesting inhibition of fibrin association as thrombin increases.²⁰⁾ Our studies showed that maximum strength was acquired using 25 units/ml thrombin concentration, a slightly lower concentration. The use of gelatin sponge as a vehicle for the fibrin glue in our study may explain this discordance.

In the clinical setting, more than 5 seconds is sometimes required from soaking of gelatin sponge with fibrinogen then with thrombin to the final positioning of the FGGS on the defect, because shifting of the FGGS on the defect is usually required to achieve adequate sealing, and even repositioning is occasionally needed. This study shows adhesion strength was not significantly different between the incubation time of 5 seconds and 20 seconds, but decayed in 60 seconds. Therefore, the neurosurgeon has at least 20 seconds lag-time from the start of conjugation of fibrinogen and thrombin on the gelatin sponge until final positioning on the defect with maximum adhesion strength provided by the 1:10 dilution of thrombin.

The rate of the postoperative CSF rhinorrhea needing second reconstruction in our 255 consecutive cases of transsphenoidal surgery (1.6%) appears lower than those of the preceding reports, 1.5–10%,^{5–7,9,10,14,17)} and suggests the usefulness of FGGS with diluted thrombin solution in neurosurgical practice. However, the rate may be influenced by the radicality of the surgery for suprasellar lesions. Therefore, a prospective comparative clinical trial between standard and diluted thrombin solution is required, followed by a pathological study to identify the factors contributing to adhesion strength.

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Conflicts of Interest Disclosure

The authors have no personal financial or institutional interest in any of the drugs, materials, or devices in the article. All authors who are members of The Japan Neurosurgical Society (JNS) have registered online Self-reported COI Disclosure Statement Forms through the website for JNS members.

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