Experimental Study of Intraparenchymal Fibrinogen and Topical Thrombin to Seal Pleural Defects

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Objective: Fibrin sealants are used to close surgical pleural defects, but may detach, causing a postoperative air-leak. We investigated a new means of applying fibrin glue for closing pleural defects.
Methods: Pleural defects (10-mm and 4-mm diameters, respectively) were created in swine and rats via thoracotomy. They were sealed by a) injection of a fibrinogen solution into the lung parenchyma after instillation of a thrombin solution onto the pleural defect (group A), b) fibrinogen and thrombin spray (group B), c) fibrinogen instillation and a thrombin-dipped polyglycolic acid sheet (group C), or d) fibrin glue-coated collagen fleece (group D). Resistance to airway pressure was compared and the sealed areas were histologically examined.
Results: In group A, the minimum seal-breaking airway pressure was consistently > 40 cmH_2O, versus 37.2 ± 3.6 cmH_2O in group B, 37.2 ± 4.0 cmH_2O in group C, and 39.0 ± 1.7 cmH_2O in group D, which was statistically significant. Histologically, the fibrin layer infiltrated the lung parenchyma and covered the defect in group A, but not in the other groups.
Conclusions: The intraparenchymal injection of fibrinogen combined with instillation of thrombin created an effective fibrin layer associated with early pleural regeneration that reliably prevented pleural air leaks.

Key words: air leak, fibrin glue, pleural defect repair, sealant

INTRODUCTION

A prolonged air-leak from a pleural fistula may be a source of major physical and mental morbidity and cause lengthy postoperative hospitalization [1]. Pleural defects are usually sutured, sealed with fibrin glue, or patched with a biocompatible sheet, singly or in combination [2–9]. However, sutures might tear the pleura, while fibrin or sheets might detach from it when the airway pressure increases. We examined, in animal models, an improved pleura-sealing technique, using a thrombin solution instilled onto the pleural defect, followed by the injection of a fibrinogen solution under the defect, into the lung parenchyma, resulting in formation of a fibrin layer that sticks to the pleural defect (Fig. 1).

MATERIALS AND METHODS

Animals

The investigation conformed to *The Guide for the Care and Use of Laboratory Animals* published by the U.S. National Institutes of Health (NIH Publication No. 85–23, revised 1996). All procedures described in this report were approved by our institutional review board for animal studies.

We used specific-pathogen free, female swine, 3–5 months of age, weighing 30 kg and, for long-term histological studies, specific-pathogen free, 10-week-old male Wistar rats (Charles River Breeding Laboratories, Tokyo, Japan), weighing 300–350 g.

Tissue sealants

The sealants used were Bolheal® fibrin glue (The Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan) and Tachosil® fibrin glue-coated collagen fleece (CSL Behring Co., King of Prussia, PA, USA). The absorptive polyglycolic acid (PGA) sheet was a 0.15-mm thick, non-woven, loose, Neoveil® highly elastic sheet (Gunze Ltd., Kyoto, Japan).

Pleural defect models

The swine were intubated, artificially ventilated, and underwent a right thoracotomy, under general anesthesia. With the airway pressure kept at 10 cm H_2O, an 8-mm diameter and 3-mm deep pleural defect was created on the surface of the anterior, middle, and posterior lung lobes, using an 8.0-mm diameter Skin Biopsy Punch, (Kai Industries Co., Ltd. Tokyo, Japan) and scissors. Hemorrhagic areas were cauterized as needed.

The pleural defect was sealed with a) a 0.2-ml drop instillation of thrombin solution (250 U/ml), followed

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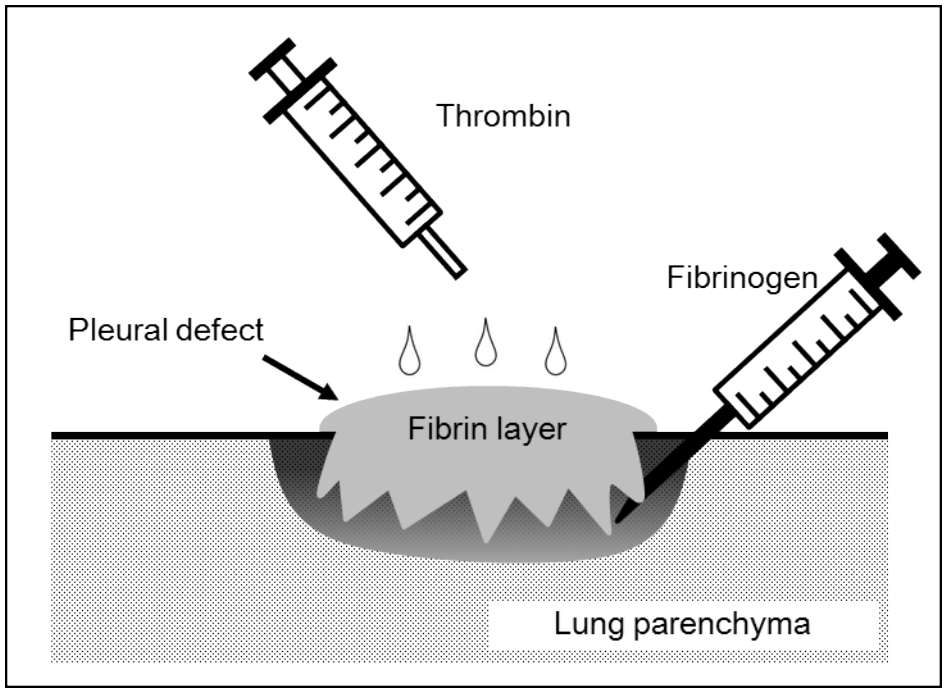

Fig. 1 Schematic representation of the technique used to seal a visceral pleural defect by the intraparenchymal injection of a fibrinogen solution beneath the defect, combined with the prior instillation of a thrombin solution on its surface, to form an infiltrating fibrin layer.

by 0.2 ml of fibrinogen solution (80 mg/ml) (group A) injected with 30-gauge needles into the lung parenchyma beneath the defect (Fig. 1), b) a simultaneous spray of 0.2-ml thrombin and 0.2 ml fibrinogen solution (group B), c) a 0.2 ml instillation of fibrinogen solution and a patch of thrombin-dipped PGA sheet (group C), or d) a fibrin-glue-coated collagen fleece (group D). The details of the methods used in groups B, C and D have been described previously [9, 10]. The lowest airway pressure that broke the seal was measured at 10 min after application of the sealant and was compared among the groups. The swine were then sacrificed and specimens of lung tissue were harvested for histological examinations.

In separate experiments, rats were intubated, artificially ventilated, and underwent left thoracotomy under general anesthesia. After clamping of the hilum with a forceps, a 4-mm diameter and 1-mm deep pleural defect was created on the surface of the left lung, using a 4.0-mm in diameter Skin Biopsy Punch (Kai Industries Co., Ltd., Tokyo, Japan) and scissors. The pleural defect was sealed, using the method used in group A above, i.e., a 0.05-ml instillation of thrombin solution followed by the injection of 0.05 ml of fibrinogen solution into the lung parenchyma. Lung specimens were harvested for histological examination, the chest was closed, and the rats were extubated and allowed to recover.

Histology

All pigs from the 4 experimental groups were sacrificed after measurement of the seal-breaking airway pressure. The rats were sacrificed 2 or 14 days after they had undergone treatment with method A. The lung specimens were inflated with 10% buffered formalin at a pressure of 10 cmH_2O, fixed, embedded in paraffin, cut into 4-μm-thick slices, stained with hematoxylin and eosin, and used for time-course analyses.

Statistical analyses

The airway pressures are reported as means ± SD. Between-group differences were examined by Student's *t*-test. A P value < 0.05 was considered statistically significant. The analyses were performed using StatView-J, version 5.0 (Abacus Concepts Inc., Berkeley, CA, USA).

RESULTS

Minimum seal-breaking pressure

The seal-breaking airway pressures were compared among the 4 swine groups (Fig. 2). The normal pleura detached diffusely from the lung parenchyma when the airway pressure was > 40 cmH_2O. Therefore, when the airway pressure reached 40 cmH_2O before the seal was broken, the value was recorded as 40 cmH_2O. In group A, all the seal-breaking airway pressures were 40 cmH_2O, in contrast with 37.2 ± 3.6 cmH_2O in group B, 37.2 ± 4.0 cmH_2O in group C, and 39.0 ± 1.7 cmH_2O in group D. Group A differed statistically significantly from the other groups (Fig. 2).

Histological examinations

On histologic examination of the swine tissue specimens (Fig. 3), the fibrin layer used to cover the pleural defect in group A was seen to have infiltrated the lung parenchyma beneath the defect (Fig. 3A), whereas in groups B, C, and D (Fig. 3B, C, & D) the fibrin or sealants were in immediate contact with the pleural defects, and it was observed that the sealants had par-

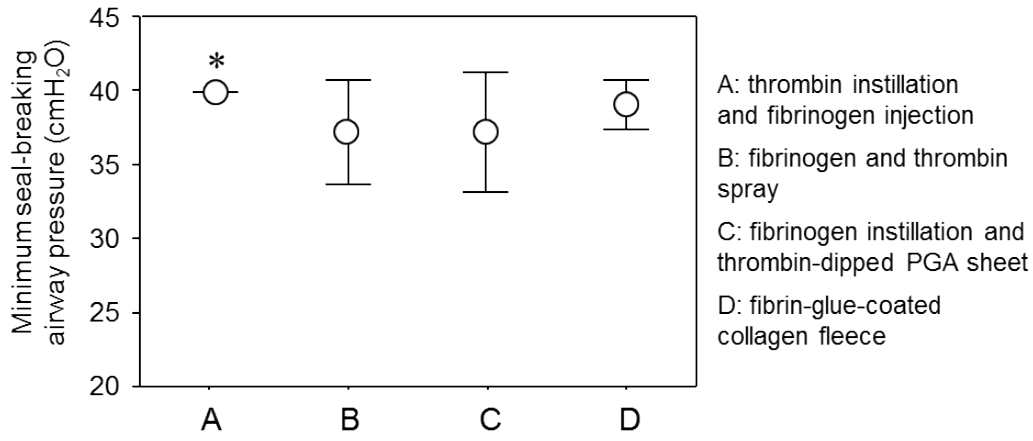

Fig. 2 Mean (± SD) minimum seal-breaking airway pressure measured 10 min after sealing a pleural defect in swine with A) a thrombin instillation followed by intraparenchymal fibrinogen injection, B) fibrinogen and thrombin spray, C) fibrinogen instillation and thrombin-dipped polyglycolic acid PGA sheet, and D) a fibrin glue-coated collagen fleece. N = 10 in each group. *$P < 0.05$, A vs. B, C, and D; Student's *t*-test.

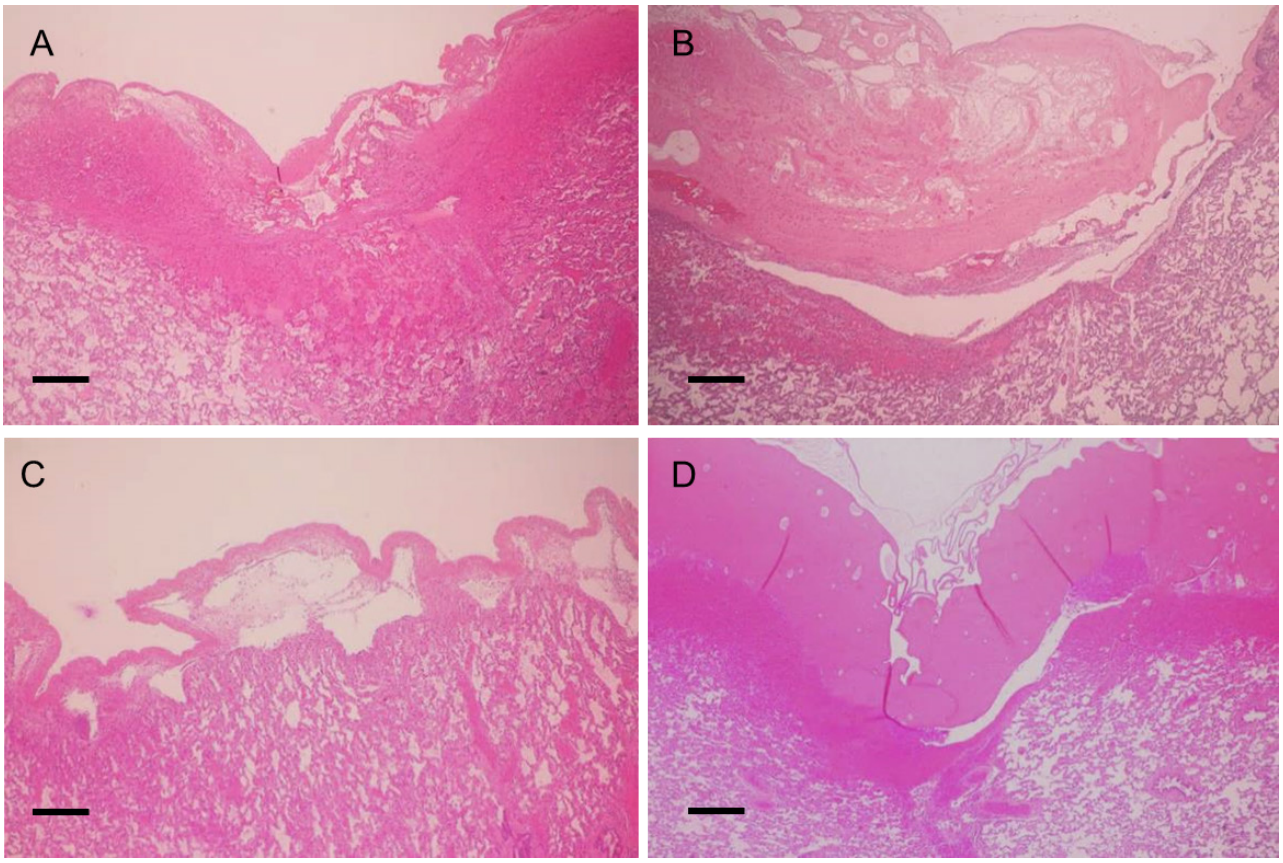

Fig. 3 Representative histology of swine lungs 10 min after seal of the pleural defect by A) thrombin instillation followed by fibrinogen injection into the lung parenchyma, B) fibrinogen and thrombin spray, C) fibrinogen instillation and thrombin-dipped polyglycolic acid PGA sheet, and D) fibrin-glue-coated collagen fleece. The fibrin layer covering the pleural defect in group A infiltrated the lung parenchyma beneath the defect, whereas in groups B, C, and D the fibrin or sealants were in immediate contact with the pleural defects and exfoliated partially. Bars = 1 mm. Hematoxylin and eosin stain.

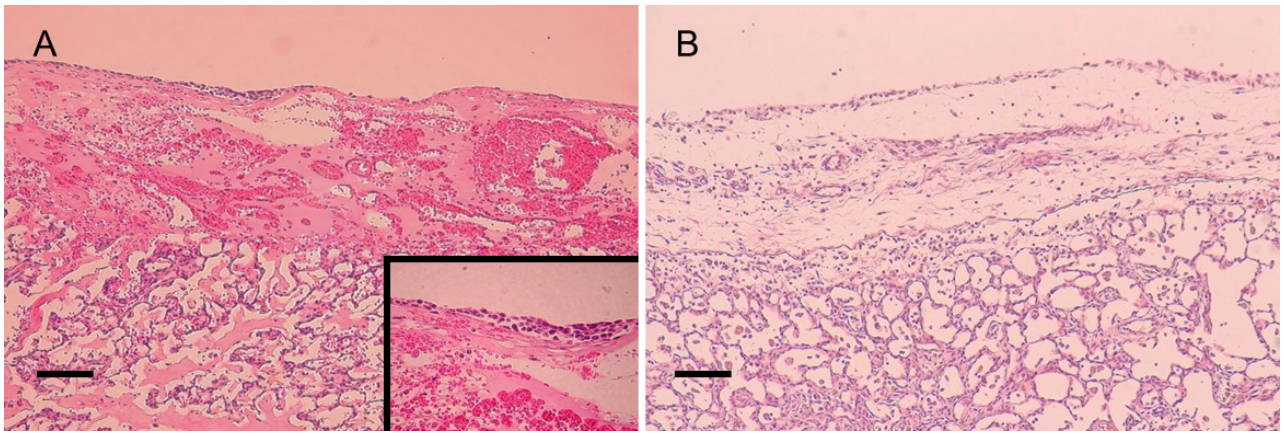

Fig. 4 Representative histology of rat lungs 2 (A) and 14 (B) days after sealing of the pleural defect by an intraparenchymal fibrinogen injection combined with the prior instillation of thrombin. A layer of mesothelial-like cells was visible on the surface of the fibrin layer within 48 h after treatment (A, inset). Thickened pleura regenerated 14 days after treatment. Bars = 50μm. Hematoxylin and eosin stain.

tially exfoliated.

In the rat histological preparations, a layer of mesothelial-like cells was visible on the surface of the fibrin layer within 48 h after treatment with method A (Fig. 4A). The defect was healed and covered with a thick pleura by 14 days after treatment (Fig. 4B).

DISCUSSION

Here, we investigated the efficacy of an improved technique of applying fibrin glue in eliminating air-leaks from pleural defects in animal models. The injection of fibrinogen combined with an instillation of thrombin applied to the pleural defects formed a fibrin layer that offered superior resistance to airway pressure as compared with other techniques or sealants. In this approach, histology showed that the fibrin layer infiltrated the lung parenchyma under the pleural defect.

Several techniques have used fibrin glue to seal pleural defects that occur after surgical dissection of pleural adhesions or interlobar fissures [4, 8–10]. However, the fibrin layer may become partially or totally detached, losing its sealing effect, when the airway pressure is increased and the lung is inflated. With most previously reported techniques, fibrinogen and thrombin are simply dribbled or sprayed onto the pleural surface, allowing the fibrin layer to separate from the defect easily.

In preliminary experiments, we found that the injection of both thrombin and fibrinogen forms a fibrin layer that is attached to the lung parenchyma, which is then capable of stopping the air-leak. However, if thrombin is infused inside the pulmonary vessels, it may cause thrombosis or severe inflammation. Therefore, injection of both thrombin and fibrinogen is not recommended for clinical use. In the present study, we found that injection of fibrinogen combined with the instillation of thrombin creates a fibrin layer that adheres to the lung parenchyma as well as after injection of both these substances. The injected fibrinogen is absorbed by the lung parenchyma and transferred to the pleural defect, where the pressure is lower than inside the lung. When in contact with the penetrating thrombin, a polymerized fibrin layer is formed that covers the pleural defect and that remains attached when the airway pressure is raised.

The early appearance of a layer of mesothelial-like cells observed histologically in the rat model may represent the early regeneration of mesothelium. However, since there is no reliable marker for rat mesothelial cells, we could not confirm this observation. This cell layer may help to maintain the air tightness of the fibrin layer. Since thrombin accelerates the proliferation and chemotaxis of mesothelial cells, it may also accelerate the regeneration of pleural mesothelium [11].

In conclusion, a new means of applying fibrin glue, consisting of the instillation of thrombin followed by injection of fibrinogen at the site of pleural defects, offered a superior resistance to airway pressure. This simple procedure may be an effective means of stopping air-leaks due to postoperative pleural defects.

CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to disclose.

REFERENCES

1) Wright CD, Wain JC, Grillo HC, Moncure AC, Macaluso SM, Mathisen DJ. Pulmonary lobectomy patient care pathway: a model to control cost and maintain quality. Ann Thorac Surg 1997; 64(2): 299–302.
2) Porte HL, Jany T, Akkad R, Conti M, Gillet PA, Guidat A, *et al.* Randomized controlled trial of a synthetic sealant for preventing alveolar air-leaks after lobectomy. Ann Thorac Surg 2001; 71(5): 1618–22.
3) Wain JC, Kaiser LR, Johnstone DW, Yang SC, Wright CD, Friedberg JS, *et al.* Trial of a novel synthetic sealant in preventing air-leaks after lung resection. Ann Thorac Surg 2001; 71(5): 1623–8.
4) Fabian T, Federico JA, Ponn RB. Fibrin glue in pulmonary resection: a prospective, randomized, blinded study. Ann Thorac Surg 2003; 75(5): 1587–92.
5) Macchiarini P, Wain J, Almy S, Dartevelle P. Experimental and clinical evaluation of a new synthetic, absorbable sealant to reduce air-leaks in thoracic operations. J Thorac Cardiovasc Surg 1999; 117(4): 751–8.
6) Herget GW, Kassa M, Riede UN, Lu Y, Brethner L, Hasse J. Experimental use of an albumin-glutaraldehyde tissue adhesive for sealing pulmonary parenchyma and bronchial anastomoses. Eur J Cardiothorac Surg 2001; 19(1): 4–9.
7) Saito Y, Omiya H, Shomura Y, Minami K, Imamura H. A new

bioabsorbable sleeve for staple-line reinforcement: report of a clinical experience. Surg Today 2002; 32(4): 297–9.
8) Miyamoto H, Futagawa T, Wang Z, Yamazaki A, Morio A, Sonobe S, *et al*. Fibrin glue and bioabsorbable felt patch for intraoperative intractable air-leaks. Jpn J Thorac Cardiovasc Surg 2003; 51(6): 232–6.
9) Gika M, Kawamura M, Izumi Y, Kobayashi K. The short-term efficacy of fibrin glue combined with absorptive sheet material in visceral pleural defect repair. Interact Cardiovasc Thorac Surg 2007; 6(1): 12–5.
10) Kawamura M, Gika M, Izumi Y, Horinouchi H, Shinya N, Mukai M *et al*. The sealing effect of fibrin glue against alveolar air-leakage evaluated up to 48 h; comparison between different methods of application. Eur J Cardiothorac Surg 2005; 28(1): 39–42.
11) Hott JW, Sparks JA, Godbey SW, Antony VB. Mesothelial cell response to pleural injury: thrombin-induced proliferation and chemotaxis of rat pleural mesothelial cells. Am J Respir Cell Mol Biol 1992; 6(4): 421–5.