EFFECTIVENESS OF CROSS-LINKED GELATIN GLUE IN CANINE LUNG SURGERY MODELS

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Abstract:

Background. Air leakage is a common postoperative complication in pulmonary surgery, and surgical sealants have been developed to prevent or reduce the incidence of air leaks. In this study, we evaluated the efficacy of cross-linked gelatin glue (gelatin plus glutaraldehyde) in canine lung surgery models.

Methods. Pulmonary fistulas and injuries were created in dogs and sealed with gelatin glue, fibrin glue, or fibrin glue with a polyglycolic acid (PGA) sheet. Seal-breaking pressures were measured in the fistula model, and pleural adhesions were assessed 28 days postoperatively in the lung injury model.

Results. The seal-breaking pressures for canine cadaver and living lung surgeries (; the maximum pressures were 80 and 40 cm H_2O) were respectively: gelatin glue, 77 ± 6 and 32.3 ± 8.9cm H_2O; fibrin glue using spray, 39.2 ± 9.3 and 32 ± 6cm H_2O; fibrin glue using the rub-and-soak method, 35 ± 13.4 and 40 ± 0 cm H_2O; and fibrin glue with a PGA sheet, 55.5 ± 18.2 and 39 ± 2cm H_2O. In the lung injury model, there were no chest wall adhesions in the gelatin and fibrin glue alone groups, while strong adhesions were observed when treated with fibrin glue with a PGA sheet.

Conclusions. Gelatin glue's sealing effect was superior to that of fibrin glue while preventing postoperative pleural adhesions. These findings suggest that gelatin glue may be effective as a surgical sealant or anti-adhesion material in lung surgery.

Key words: gelatin, biomaterial, lung surgery, surgical sealant, anti-adhesion

Introduction

In lung surgery, the most popular surgical sealant is fibrin glue, which has been used to prevent air leaks over the last twenty years[1–5]. Although fibrin glue does reduce postoperative air leaks[6], it has some disadvantages, including bonding strength inadequacy, which is

associated with postoperative air leaks[7], and the fact that it is a blood product. Because fibrin glue alone is considered inadequate for stopping air leaks, a polyglycolic acid (PGA) sheet (Neoveil, GUNZE Ltd., Kyoto, Japan) is often used in conjunction with fibrin glue in Japan. Although this combination has been reported to be more effective than fibrin glue alone[8–10], we have sometimes observed air leaks after lung surgery even when fibrin glue was used with a PGA sheet. In addition, there are concerns that this combination may induce pleural adhesions [11, 12].

Our laboratory has created a biological glue composed of gelatin and glutaraldehyde (cross-linked gelatin glue, hereafter referred to as gelatin glue) with high adhesive strength and good biocompatibility, as described previously[13]. Using a rat lung surgery model, we previously demonstrated that this gelatin glue had a bonding strength two- to three-fold higher than that of fibrin glue[14] and that it inhibited pleural adhesions[15]. In this study, we investigated the sealing effect and occurrence of pleural adhesions associated with this gelatin glue relative to fibrin glue (with or without a PGA sheet) in canine lung surgery models to assess the feasibility of clinical application.

Materials and Methods

Materials

Gelatin glue was prepared and mixed as previously described[13–15]. Briefly, the glue was "prepared by mixing a 26% (w/v) aqueous solution of gelatin (alkaline-processed gelatin, MW 89,000; Nippi, Tokyo, Japan) and a 1% (w/v) glutaraldehyde solution (mix ratio; 100 : 13) (both sterile)." It was heated at 45℃ using the heating applicator, and 38℃ at the time extruded onto the tissue surface. Fibrin glue (Beriplast P Combi-Set; CSL Behring, Tokyo, Japan) was purchased from Waken Co. Ltd. (Japan), and PGA sheets (Neoveil; 0.15 mm thick) were kindly supplied by GUNZE Ltd. Female beagle dogs (8–12 months old, weighing approximately 9–12 kg) were purchased from Japan Oriental Bio Service, Inc. (Kyoto, Japan). All animal housing, care, and surgical procedures were performed in accordance with the institutional guidelines of the animal research committees of Nara Medical University.

Measurement of Seal-Breaking Pressures in Canine Cadaver Lungs

Endotracheal intubation was performed and a respirator was connected (Model CT-7FII, ACOMA Medical Industry Co., Ltd., Tokyo, Japan). A wound sufficient to cause air leaks was created as previously described, by "pricking the left lung using a 23-guage needle to a depth of 2 mm from the lung surface"[14]. The air leak was confirmed by bubble formation at the wound site when the lung was manually inflated. Gelatin glue (n=5), fibrin glue by spray (n=6), fibrin glue by rub-and-spray (n=5), or fibrin glue by rub-and-soak with a PGA sheet (10mm × 10mm, n=6) was applied over the pulmonary puncture wound. The volume of glue applied was 0.2 ml for both gelatin and fibrin glues as previously described.[14] The rub-and-soak method for fibrin glue was performed according to the previously published protocol, and this method was used for application with a PGA sheet[9]. We waited for 5 min after application for the glues to solidify and adhere to the lung surface. To measure the seal-breaking pressure, the lung was inflated manually at a constant rate through the endotracheal tube (maximum pressure limit of 80cm H_2O), and air leakage was identified by observing bubble formation from the sealed

needle puncture site. The pressure in the respiratory tract at which air leakage occurred was recorded for each material. After measurement, the treated area was isolated with a surgical clamp forceps, and the same procedure was performed on a different area of the lung. This was repeated 5–6 times for each canine cadaver; one type of glue was applied for each dog.

Measurement of Seal-Breaking Pressures in Canine Living Lungs

Dogs were given intraperitoneal injections of pentobarbital sodium (30mg/kg) and placed on the surgical table with an electric blanket to maintain body temperature. Endotracheal intubation was then performed and a respirator was connected (Model CT-7FII, ACOMA Medical Industry Co., Ltd.). Dogs were ventilated at ≤ 20 cm H2O. When stable respiration was established, a lateral thoracotomy was performed in an intercostal space. An air-leaking wound was created by pricking the lung surface using a 23-guage needle to a depth of 2 mm from the lung surface, as described previously (Figure 1, A and B). Hemostasis was achieved by pressing with cotton gauze, and the air leak was confirmed by observing bubble formation at the wound site when the lung was manually inflated. After artificial respiration was stopped, gelatin glue (n=5) (Figure 1C), fibrin glue by spray (n=6), fibrin glue by rub-and-spray (n=5), or fibrin glue by rub-and-soak with a PGA sheet (10mm × 10mm, n=6) was applied over the pulmonary puncture wound. The volume of glue applied was 0.2 ml for both gelatin and fibrin glues as described previously. The waiting process after covering the wound was performed as described previously (1 min after application, artificial respiration was started again and maintained for 4 min)[14]. To measure the seal-breaking pressure, the lung was inflated manually at a constant rate through the endotracheal tube (maximum pressure limit of 40cm H_2O), and air leakage was identified by observing bubble formation from the sealed needle puncture site (Figure 1D). The pressure in the respiratory tract at which air leakage occurred was recorded for each material. This procedure was then repeated 5–6 times for each dog, as described previously. Finally, dogs were euthanized with overdoses of pentobarbital sodium (250mg/kg).

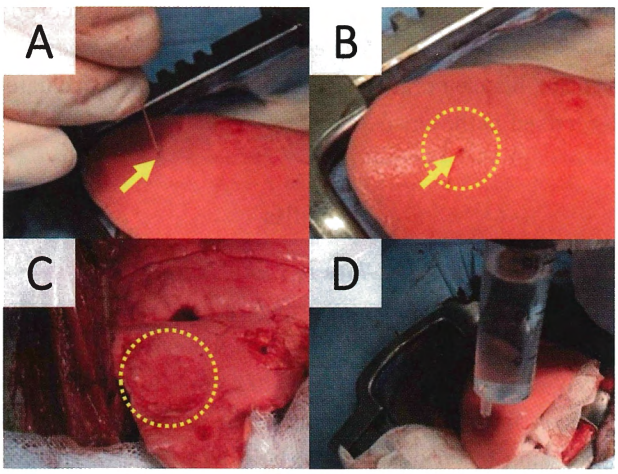

Figure 1. Photographs showing the experimental procedure for sealing the wound in the canine pulmonary fistula model. (A) The pulmonary fistula was created in the lung using a 23-gauge needle inserted to a depth of 2 mm from the surface. (B) Needle hole. (C) The fistula sealed with glue (0.2 ml); gelatin glue was used in this photograph. (D) The seal-breaking pressure was measured using a water bubble.

Preparation of Canine Lung Injury Model to Assess Intrapleural Adhesion

After induction of general anesthesia and initiation of ventilation, a lateral thoracotomy was performed as described previously. A wound (5–10 mm long, 1mm deep) was then created on the lung surface using surgical scissors. Hemostasis was achieved by pressing with cotton gauze for a few minutes. Artificial respiration was then stopped before applying gelatin glue (n=5), fibrin glue (n=5), or fibrin glue with a PGA sheet (25 mm × 25 mm, n=5) over the pulmonary wound. One minute after application, artificial respiration was restarted and maintained for 4 min, and the chest was closed (Figures 2, 3). Twenty–eight days after surgery, the treated area was exposed by lateral thoracotomy under general anesthesia and assessed for adhesions by a thoracic surgeon who was blind to the treatment group.

Randomization of Groups, Measurement of Seal-Breaking Pressure, and Statistical Analyses

The four different sealants were randomly allocated to one canine cadaver or living lung, with 5–6 lesions and one type of sealant per lung. All seal–breaking values were mean ± standard deviation. The results were statistically analyzed by Kruskal–Wallis test using GraphPad Prism software (Version 6.0). All p–values less than .05 were considered to indicate statistical significance. No statistical power calculations were undertaken. Observer blinding during measurement of seal–breaking pressures was not possible because of obvious differences in product appearance.

Evaluation of Adhesion Model

The three different sealants were randomly allocated to the dogs, with one lesion and one sealant per dog. No statistical power calculation was undertaken. A thoracic surgeon who was blind to the treatment groups assessed adhesions 28 days postoperatively.

Histologic Examination of Adhesion Model

The lung, including the surgical site, was subsequently resected and fixed in 10% formaldehyde aqueous solution. The fixed specimens were cross–sectioned and stained with hematoxylin and eosin for histologic analysis. The wounds were evaluated histologically and assessed by a board–certified pathologist.

Results

Bonding Strength of Gelatin and Fibrin Glues to Lung Tissue

The photographs in Figure 1 show the experimental procedure for measuring the bursting pressure of the pricked lung sealed with the various materials. Both gelatin and fibrin glues adhered to the canine lung surface immediately after application. The gelatin glue responded promptly to lung expansion and contraction owing to the glue's inherent elasticity.

In the canine cadaver lung experiment, the gelatin glue adhered firmly to the lung surface and prevented air leakage, withstanding a pressure of over 70cm H_2O. The gelatin glue had a significantly higher seal–breaking pressure (77 ± 6cm H_2O) than the fibrin glue alone groups (spray, 39.2 ± 9.3cm H_2O; rub–and–soak method, 35 ± 13.4cm H_2O) or the fibrin glue with a

PGA sheet (55.5 ± 18.2 cm H2O). The p-values for comparisons between the gelatin and fibrin (spray) groups and the gelatin and fibrin (rub-and-soak) groups were .034 and .005, respectively (Figure 4). The gelatin glue adhered strongly to the lung tissue, and it was difficult to remove the gelatin glue from the tissue using forceps.

In the canine living lung experiments, the gelatin glue adhered strongly to the lung surface and performed equivalently to the fibrin glue groups (gelatin glue, 32.3 ± 8.9cm H_2O; fibrin glue using spray, 32 ± 6 cm H_2O; fibrin glue using the rub-and-soak method, 40 ± 0cm H2O; fibrin glue with a PGA sheet, 39 ± 2cm H_2O [Figure 5]). The maximum pressure for living lung experiments was set at 40cm H_2O. Six out of the eleven gelatin glues (55%) remained stably adherent to the lung at 40cm H_2O.

Anti-Adhesive Effects of Gelatin Glue in the Pleural Cavity

Figures 4 and 5 show photographs of the experimental wound-sealing procedure and the lungs 28 days postoperatively for the intrapleural adhesion model. The results for each dog in the different groups are summarized in Table 1. There were no adhesions of the chest wall in any of the dogs in the gelatin glue (Figure 2C) and fibrin glue alone groups, whereas strong adhesions were observed in all four dogs in which fibrin glue was used with a PGA sheet (Figure 3C). In the gelatin glue and fibrin glue alone groups, the glues were completely absorbed with no or only mild inflammation after 28 days (Figure 6, A-D). When fibrin glue was used with a PGA sheet, the PGA sheets remained and inflammation existed around the wound-sealing site (Figure 6, E and F).

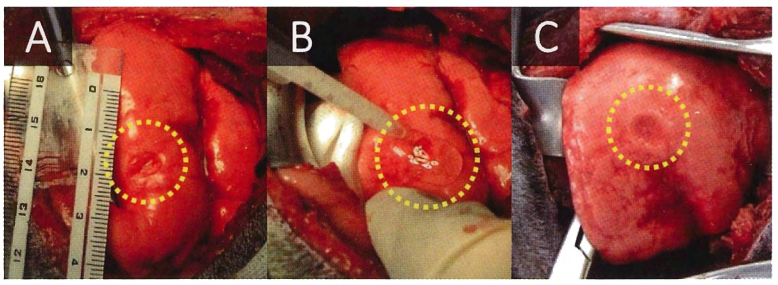

Figure 2. Experimental wound-sealing procedure in the canine pulmonary incision model. Typical examples of lung lesions sealed with gelatin glue are shown. (A) A fistula (5-10 mm) was created in the lung using surgical scissors. (B) The fistula was sealed with gelatin glue. (C) The wound appearance at 28 days postoperatively.

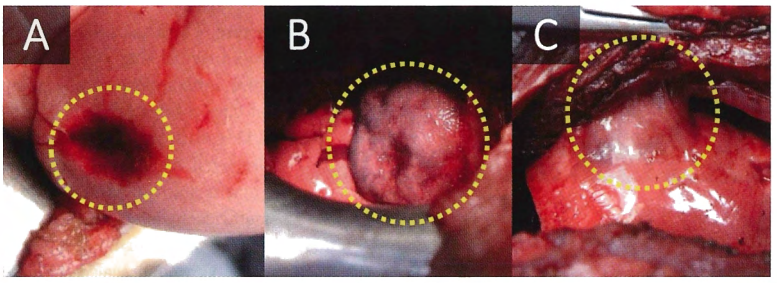

Figure 3. Experimental wound-sealing procedure in the canine pulmonary incision model. Typical examples of lung lesions sealed with fibrin glue and a polyglycolic acid sheet are shown. (A) A fistula (5-10 mm) was created in the lung using surgical scissors. (B) Fibrin glue and a polyglycolic acid sheet were applied. (C) The wound had adhered to the chest wall at 28 days postoperatively.

Table 1. Adhesions associated with injured canine lungs treated with different materials

Gelatin glue*	Fibrin glue	Fibrin glue with PGA † sheet
No adhesion	No adhesion	Severe§
No adhesion	No adhesion	Severe§
Failure ‡	No adhesion	Severe§
No adhesion	No adhesion	Severe§
No adhesion		

* Gelatin glue: 26% gelatin + 1% glutaraldehyde
† PGA, polyglycolic acid
‡ Surgery prolonged because of bleeding
§ Severe: adhesions requiring sharp dissection to separate

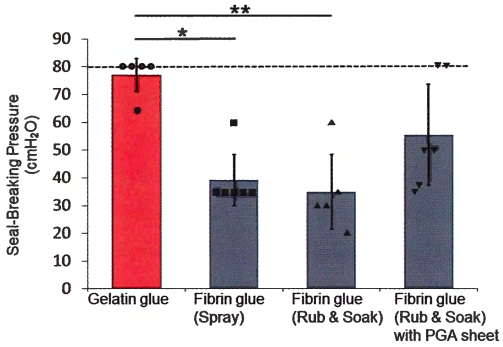

Figure 4. Ex vivo seal-breaking pressure after repair in the canine cadaver lung. Groups : gelatin glue, 77 ± 6cm H_2O; fibrin glue by spray, 39.2 ± 9.3cm H_2O; fibrin glue by rub-and-soak, 35 ± 13.4cm H_2O; fibrin glue by rub-and-soak with polyglycolic acid sheet, 55.5 ± 18.2cm H_2O. *, p=.034; **, p=.005. Maximum pressure was 80cm H_2O.

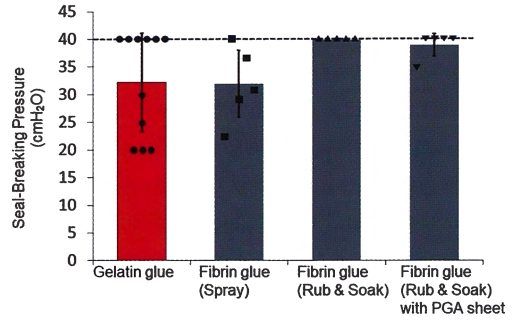

Figure 5. Seal-breaking pressure after repair in canine living lungs. Groups : gelatin glue, 32.3 ± 8.9cm H_2O; fibrin glue by spray, 32 ± 6cm H_2O; fibrin glue by rub-and-soak, 40 ± 0cm H_2O; fibrin glue by rub-and-soak with polyglycolic acid sheet, 39 ± 2cm H_2O. Maximum pressure was 40cm H_2O.

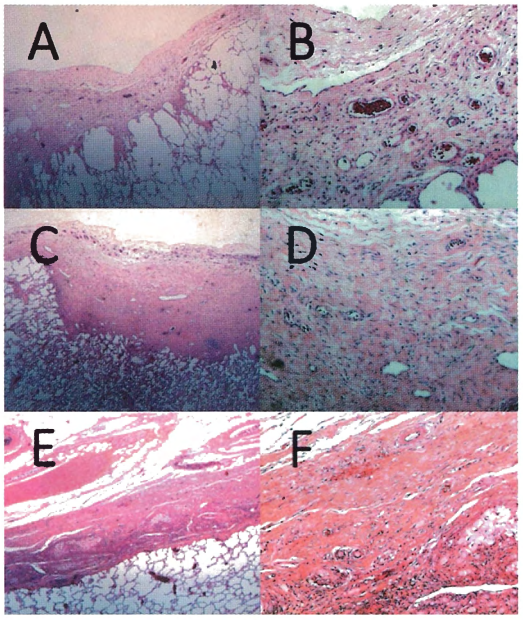

Figure 6. Histopathologic images of sealed canine lung wounds (wounds were 5-10mm long, 1 mm in depth) on day 28 after surgery. A: wound sealed with gelatin glue (0.2 mL, prepared by mixing 26% wt/vol gelatin solution and 1% wt/vol glutaraldehyde solution). B: wound sealed with fibrin glue (0.2 mL) alone. C: wound sealed with fibrin glue (0.2 mL) and a polyglycolic acid sheet (10 mm x 10 mm). Hematoxylin and eosin staining, original magnification x 50 (A, C, E).

Comment

We have previously reported the sealing and adhesion prevention effects of gelatin glue in a rat lung surgery model [14, 15]; in the current study, we used a large animal model to assess the feasibility of clinical application in humans. In this canine lung surgery model, the gelatin glue adhered well to the lung, and pleural adhesions to the adjacent chest wall were not observed 28 days postoperatively.

With regard to the seal-breaking pressure, the gelatin glue showed strong adhesion in the previous live rat experiments, with a seal-breaking pressure of 47.9 ± 6.7 mm Hg (65.1cm H_2O); this was significantly higher than the seal-breaking pressures of the fibrin glue groups (14). In this study using canine models, the gelatin glue firmly adhered to the lung surface and showed strong adhesion, withstanding high pressures. In the canine living lung experiment, the gelatin glue withstood the maximum pressure of 40cm H_2O in 6 out of the 11 cases (55%); in these cases, the glue may have stood up to seal-breaking pressures well over 40 cm H_2O. Indeed, the canine cadaver lung experiment showed a higher seal-breaking pressure of 77 ± 6cm H_2O for the gelatin glue group, which was significantly higher than that of the fibrin glue alone groups.

The canine living lung experiment showed that both the gelatin and fibrin glues had good pressure resistance, indicating that they are clinically applicable as sealants. However, although the gelatin glue always achieved seal-breaking pressures of over 60cm H_2O in the cadaver lung experiment, the seal-breaking pressures were less than 40cm H_20 in 5 out of the 11 cases (45%) in which gelatin glue was used in the living lung experiment. There are several factors related to the inherent nature of gelatin glue and the experimental setup that might have caused these results. First, the influence of body temperature on the physical crosslinking of gelatin is a relevant factor. With gelatin glue, glutaraldehyde is used to chemically crosslink the gelatin network within 30 s of mixing, and the strength of the gel increases over time. However, if the temperature of the applied surface is low, the physical crosslinking of gelatin tends to be accelerated, resulting in a stronger gel faster. Because the cadaver lung was cooler (room temperature of ~22℃ versus ~38.5 ℃ for the living canine lung), physical crosslinking would have resulted in faster gelation on the cadaver lungs. In addition, we used an electric blanket in this experiment to maintain the dogs' body temperature during the surgeries, whereas we did not use blankets in the previous rat experiment. Although the body temperature of rats (37℃) is similar to that of dogs, the surface of the rat lung might have been cooler during the surgeries because of the lack of thermal support. Second, in the living lung experiment, we had to start artificial respiration only 1 min after glue application, and this movement might have prevented good adhesion to the lung tissue in some cases; in contrast, the cadaver lungs remained still for 5 min before pressure measurements, giving enough time for the glue to anchor onto tissue surfaces. Finally, changes in the viscosity of the gelatin solution due to variations in application temperature can affect the ease of glue penetration into the damaged tissue opening before solidification.

Regarding prevention of pleural adhesions, no adhesions involving the area sealed with gelatin glue were observed in this experiment; this was also the case in the previous report involving rats[15]. The healing of the macroscopically damaged portion of the lung was good, and the gelatin glue disappeared by the 28th day after application. As we previously reported, we

believe that good tissue healing—which suppressed inflammation—prevented adhesions. In one of the dogs allocated to treatment with gelatin glue, bleeding occurred when the thoracic drain was inserted after gelatin glue application, and this case was judged to be a failure (Table 1).

There are some discrepancies with regard to seal-breaking pressures between the current canine living lung model and the previous rat experiment; in some of the live dogs, the gelatin glue did not seem to perform as well. However, the gelatin glue was able to cover the injured tissue surface well in general, and the healing of the affected areas was excellent, leading to complete prevention of tissue adhesions. Considering the overall results of the current large animal study, it is possible that a human clinical trial will also produce useful results. Additional studies assessing application conditions and the improvement of applicators to achieve better mixing and solidification will be important in the future.

This study had several limitations. First, we did not perform a power analysis prior to the study because this was a preliminary study; therefore, it was not possible to calculate the appropriate sample size. However, previous studies showed similar results using the same sample size. Accordingly, we presumed that including additional animals would not greatly change the overall results, and we therefore minimized the number of dogs used in accordance with an animal welfare standpoint. Second, observer blinding was not possible due to obvious differences in the appearance of the products. Third, we assessed wound healing at day 28 based on the rate of disappearance of the gelatin glue established in a previous study [15], but we have not assessed the effects over longer periods. These limitations should be considered when interpreting the results and conclusions of this study.

In conclusion, the gelatin glue prevented pleural adhesions and showed excellent sealing properties in canine lung surgery models. Our findings suggest that gelatin glue is a promising new biomaterial for use as a surgical lung sealant.

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Conflicts of Interest

The Thoracic and Cardiovascular Surgery unit at Nara Medical University has no financial interest in the materials or methods used, nor any financial relationship with any of the manufacturers mentioned in this report. The authors declare no conflicts of interest.

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