Sealing Effect of Cross-Linked Gelatin Glue in the Rat Lung Air Leak Model

Norikazu Kawai, MD, Shuko Suzuki, PhD, Hiroshi Naito, MD, Keiji Kushibe, MD, Takashi Tojo, MD, Yoshito Ikada, PhD, and Shigeki Taniguchi, MD

Department of Thoracic and Cardiovascular Surgery, Nara Medical University, Nara, Japan; Department of Medical Engineering, Nara Medical University, Nara, Japan; Queensland Eye Institute, South Brisbane, Queensland, Australia; Department of Thoracic Surgery, Nara Prefecture General Medical Center, Nara, Japan; and Kyoto University, Kyoto, Japan

Background. **Air leak is a common problem in pulmonary surgical procedures. In this study, we evaluated the efficacy and safety of gelatin glue (cross-linked with glutaraldehyde) in a rat model of lung air leak.**

Methods. **A model of pulmonary fistula was created in the rat lung with the use of a needle. The fistula was then sealed with either gelatin glue (group A), fibrin glue (group B), or fibrin glue with a polyglycolic acid sheet (group C). The seal breaking pressures were measured for each group, and the results were compared. To assess the biocompatibility of the gelatin glue, a model of lung damage was created with incision, and the gelatin glue was applied to seal the wound. Histologic analysis was then performed on the lung tissue.**

Results. **The seal breaking pressure in group A (47.88 ± 6.69 mm Hg) was significantly higher than that in group B (24.67 ± 3.24 mm Hg, $p = 0.0302$) or group C (28.67 ± 3.55 mm Hg, $p = 0.0406$). Histologically, the gelatin glue adhered firmly to the lung surface, and only localized tissue inflammation was observed.**

Conclusions. **The sealing effect of gelatin glue was superior to that of fibrin glue, with or without a polyglycolic acid sheet. In addition, the gelatin glue only caused mild inflammation of the lung and was absorbed without any adverse foreign body response. These findings suggest that gelatin glue may be a therapeutically effective biomaterial for sealing lung wounds and restoring respiratory function.**

(Ann Thorac Surg 2016;102:282–6)

Air leak during lung resection remains a serious complication, leading to prolonged chest intubation and hospitalization. Moreover, air leak has a detrimental effect on postoperative outcome because the protracted chest intubation is associated with greater pain, reduced mobility, and an increased risk of further complication [1]. Therefore, the prevention of air leaks has been a major clinical objective after lung operation. Currently, suturing, stapling, and sealing with surgical sealant are therapeutic options for air leak. The most popular surgical sealant for the lung is fibrin glue, and it has been applied to prevent air leaks over the past 20 years [2–6]. Although fibrin glue is associated with a positive outcome, it has several disadvantages, including potential virus transmission (because it is a blood product) and high cost. In addition, fibrin glue has been reported to yield inconsistent results [2, 3] when used for closure of intraoperative air leaks, often leading to prolonged air leak and protracted hospitalization, similar to conventional procedures. It has been reported that the bonding strength of fibrin is weak [7]. 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. This combination has been reported to be more effective than fibrin glue alone [8–10]. However, we have sometimes observed air leaks after lung operation, even when using fibrin glue with a PGA sheet.

Other biological adhesives [11–14] and synthetic biomaterials [15–19] used for pulmonary sealing have shown only marginal efficacy because of their low adhesive stability and poor biocompatibility. BioGlue (bovine serum albumin with glutaraldehyde; CryoLife, Inc, Kennesaw, GA) and TachoSil (collagen sponge with fibrin glue components; Nycomed, Linz, Austria) have shown high bonding strengths in a comparative study of lung sealants [7]. However, BioGlue has a relatively high toxicity and is not authorized for use in lung air leakage in many countries, including Japan, and TachoSil is difficult to apply to the wound, resulting in variable outcome.

Our laboratory has created a biological glue composed of gelatin and glutaraldehyde with high adhesive strength and good biocompatibility. In this study, we evaluate the efficacy and safety of this gelatin glue in a rat model of lung air leakage. We previously showed that the gelatin glue, prepared by mixing 26% (wt/vol) gelatin and 1% (wt/vol) glutaraldehyde solutions, exhibited a bonding strength almost three times that of fibrin glue [20]. In the current study, we apply this gelatin glue to seal rat lung needle puncture wounds, and we measure the seal

Accepted for publication Feb 11, 2016.

Address correspondence to Dr Kawai, Department of Thoracic and Cardiovascular Surgery, Nara Medical University, 840 Shijo-cho, Kashihara, Nara 634-8522, Japan; email: kawai30@hotmail.co.jp.

Published by Elsevier

0003-4975/$36.00
http://dx.doi.org/10.1016/j.athoracsur.2016.02.038

breaking pressure to assess effectiveness. We also compare the efficacy of the gelatin glue with that of fibrin glue, which is presently used clinically, with or without a PGA sheet. Furthermore, we perform histologic analysis to evaluate the biocompatibility of the gelatin glue.

Material and Methods

Materials

Gelatin glue was prepared by mixing a 26% (wt/vol) aqueous solution of gelatin (alkaline-processed gelatin; mol wt 89,000; Nippi, Tokyo, Japan) and a 1% (wt/vol) glutaraldehyde solution (both sterile) at 38°C (Fig 1). Fibrin glue (Beriplast P Combi-Set; CSL Behring, Tokyo, Japan) was purchased from Waken Co Ltd (Osaka, Japan), and PGA sheets (Neoveil, 0.15 mm in thickness) were kindly supplied by GUNZE Ltd. Female Wistar ST rats (8 weeks old, weighing approximately 200 g) were purchased from Japan SLC Inc (Kyoto, Japan). 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 Rat Lung Seal Breaking Pressure

Rats were given intraperitoneal injection of pentobarbital sodium (0.0162 mg/g). Intratracheal intubation was then performed with a 16-gauge polytetrafluoroethylene catheter (outer needle of BD Angiocath; BD Medical Systems, Franklin Lakes, NJ), which was fixed with 2-0 surgical suture and connected to a respirator (Model SN-480-7; Shinano Co Ltd, Tokyo, Japan). Rats were ventilated with air at a rate of 40 breaths per minute and a tidal volume of 4 mL. When stable respiration was established, left lateral thoracotomy was performed in the fifth intercostal space. An air-leak wound was created by pricking the left lung with a 23-guage needle to a depth of 2 mm from the lung surface. The air leak was confirmed by bubble formation at the wound site when the lung was manually inflated with a syringe (Figs 2 and 3). After artificial respiration was stopped, gelatin glue (group A, n = 8), fibrin glue (group B, n = 6), or fibrin glue with a PGA sheet (10 mm × 10 mm) (group C, n = 6) was applied over the pulmonary puncture wound. The volume of glue applied was approximately 0.1 mL for both gelatin and fibrin glues. Glues were solidified and fixed to the lung surface 1 minute after application, then artificial respiration was again started and maintained for 4 minutes. To measure the seal breaking pressure, the lung was inflated manually at a constant rate of 0.2 mL/s with a syringe (5 mL; Terumo Co Ltd, Tokyo, Japan) through the tracheal tube, and air leakage was identified by observing bubble formation from the sealed needle puncture wound site. The pressure in the respiratory tract at which air leakage occurred was recorded for each glue.

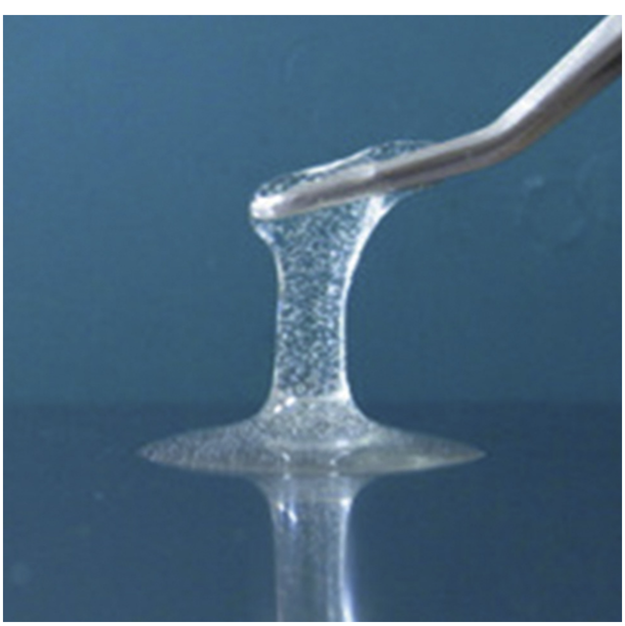

Fig 1. *Gelatin glue cross-linked with glutaraldehyde.*

Randomized Grouping

The three different sealants were randomly allocated to 20 rats, with one lesion and one sealant per rat. Each sealant was applied to six or eight rats, based on previous studies. No statistical power calculation was undertaken. Observer blinding was not possible because of obvious differences in product appearance.

Histologic Examination

For examining tissue reactivity to the gelatin glue, a 5-mm-long incision, 1 mm deep, was created in the left lung of a rat with the use of surgical scissors, and air leaks were confirmed by appearance of air bubbles. After hemostasis by pressing with cotton balls for a few minutes, the gelatin glue (0.2 mL) was applied over the wound, as described previously, and the chest was closed. On postoperative days 3, 7, and 14, the rats were euthanized with an overdose of anesthesia (pentobarbital, 0.3 mg/g), and a left-lateral thoracotomy was performed to examine the treated area (n = 3 for each time point). The left lung, including the surgical site, was subsequently resected and

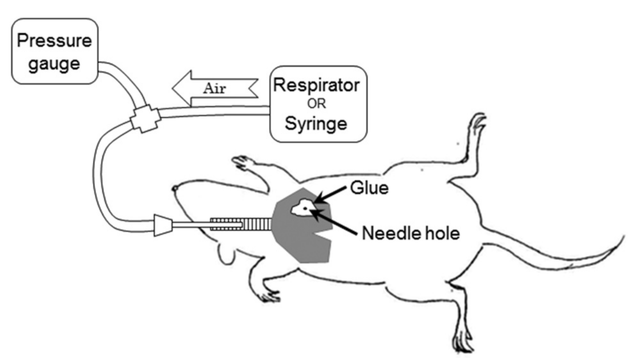

Fig 2. *Schematic representation of the experimental setup for measuring the bursting pressure of the needle-punctured and glue-sealed lung (puncture created with a 23-gauge needle inserted to a depth of 2 mm).*