Creation of a uniform pleural defect model for the study of lung sealants

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Objective: Animal models are indispensable for the development of new therapeutic methods for the closure of alveolar air leakage. However, it is difficult to create a uniform pleural defect model. The purpose of this study was to establish an appropriate animal model for assessing the efficacy and histotoxicity of synthetic sealants for lung surgery.

Methods: Nine beagle dogs were used to evaluate the pleural defect model in comparison with conventional resection procedures. A donut-shaped silicon ring with an inner diameter of 15 mm was placed on the pleura, and 0.1 mL of cyanoacrylate was dropped into the ring. A pleural defect was created by sliding a microtome blade just beneath the polymerized cyanoacrylate. Hemostasis was performed by pressure with a sponge.

Results: Morphologically, round areas of the pleura were uniformly resected with our procedure. The resected tissue consisted of pleura and thin underlying lung parenchyma. Among the results from 3 surgeons, there were no significant differences in the mean time required for hemostasis ($P = .69$), the mean thickness of the resected tissue ($P = .13$), and the mean amount of air leakage from the resected area ($P = .19$). No penetration of cyanoacrylate into the lung parenchyma was evidenced by immunofluorescence microscopy. Histologically, when the pleura was resected without using cyanoacrylate, a thick fibrocellular layer extended to the lung parenchyma. Furthermore, severe fibrosis was observed when electrocautery was used for hemostasis. However, when the pleura was resected using cyanoacrylate, the normal alveolar structure was preserved.

Conclusions: Our uniform pleural defect model using cyanoacrylate may be feasible for the evaluation of synthetic sealants for alveolar air leakage.

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Postoperative alveolar air leakage is a common complication of lung surgery. Although this complication is usually not life-threatening, management of such an air leakage requires a chest tube, which causes increased pain and morbidity and longer hospitalization. Various tissue sealants have been applied to prevent air leakage after lung surgery,¹⁻⁵ and recently some new synthetic sealants were developed and experimentally evaluated using animal models.⁴⁻¹⁴

It is indispensable to create a uniform pleural defect for evaluating the characteristics of sealants, such as the mean seal pressure and histologic changes in the lung parenchyma after application. Previous attempts to create an experimental model with a pleural defect have been performed by resecting the lung surface with scissors, scalpels, or blades.¹⁰⁻¹⁴ However, it was difficult to create pleural defects of uniform size and depth because of the characteristics of the lung, which has soft tissue, a round shape, and a smooth surface. Furthermore, hemostasis after pleural resection was often obtained with the use of electrocautery, which causes thermal

Abbreviations and Acronyms

FITC = fluorescein-4-isothiocyanate

injury of the lung.^{14,15} There have been no detailed assessments of the histologic effects on the lung because of the thermal injury resulting from electrocautery.

We developed a beagle model in which a uniform pleural defect was created using a silicone ring and cyanoacrylate to evaluate the correct seal pressure and histologic changes after the application of synthetic sealants. Our method made it possible to resect an area of the pleura with uniform size and thickness because of instant flattening of the lung surface by the powerful adhesive qualities and instantaneous polymerization of cyanoacrylate.

The purpose of this study was to establish an experimental pleural defect model for assessing the effectiveness and histotoxicity of synthetic sealants for the closure of alveolar air leakage. The uniformity of the pleural defects and histologic changes were investigated in comparison with conventional resection procedures.

Materials and Methods**Animals and Anesthesia**

Nine adult beagle dogs, weighing 8 to 12 kg, were used for this study. The animals were housed individually and provided food and water ad libitum. At the end of the experiment, the animals were euthanized by intravenous administration of an overdose of pentobarbital sodium. All the experiments were performed in

accordance with the "Principles of Laboratory Animal Care" advocated by the Animal Experiment Committee of Kyoto University (1989).

After premedication with atropine sulfate at 0.03 mg/kg, the dogs were anesthetized by intramuscular injection of ketamine hydrochloride at 15 mg/kg and xylazine at 7 mg/kg. After intratracheal intubation, mechanical ventilation was started at a respiratory rate of 14 breaths/min and a tidal volume of 25 mL/kg (50% oxygen, 50% nitrous oxide mixed with 1% halothane) to allow maintenance of anesthesia by inhalation during surgery. The airway pressure was sustained at 10 to 15 cm/H₂O.

Procedures to Create Pleural Defects Using Cyanoacrylate

The animals were placed in the right decubitus position, and a left thoracotomy was performed. A silicone donut-shaped ring with an inner diameter of 15 mm, an outer diameter of 30 mm, and a thickness of 1 mm was placed on the dry lung surface, which had been wiped with a sponge; then, 0.1 mL of *n*-butyl cyanoacrylate (ALTECO Inc, Osaka, Japan) was dropped into the center of the ring while the airway pressure was held at 20 cm/H₂O. After the cyanoacrylate had almost fully polymerized in approximately 30 seconds, mechanical ventilation was resumed. The silicone ring was removed 60 seconds after application without breaking the polymerized cyanoacrylate. A microtome blade (S35: blade thickness 250 μ m, Feather Safety Razor Co, Ltd, Osaka, Japan) was then slid just beneath the strongly adhering cyanoacrylate while the airway pressure was held at 20 cm/H₂O. After resection of the pleura in this way, hemostasis was performed by pressure with a sponge (Figure 1).

Figure 1. Our resection procedure using a donut-shaped silicone ring, cyanoacrylate, and a microtome blade. A, The silicone ring was placed on the dry lung surface, and cyanoacrylate was dropped into the center of the ring. B, The silicone ring was removed. C, A blade was slid just beneath the strongly adherent cyanoacrylate. D, Lung in which hemostasis was obtained by sponge pressure after pleural resection.

Figure 2. Quantification of air leakage from the pleural defect using the collection over water technique.

Assessment of Uniformity of the Resected Pleura

Three beagle dogs underwent this procedure performed by 3 respiratory surgeons to evaluate the uniformity of the pleural defects. Ten lesions were created on the left lung per animal, 5 each in the upper and lower lobes, so that they were located well away from each other. The shapes of the resected tissues were observed macroscopically, and the time required for hemostasis by sponge pressure was recorded for each lesion. Air leakage was tested under water while the airway pressure was held at 15 cm/H₂O to confirm whether there was significant air leakage from the whole of the resected area. The resected tissue was fixed in 10% formalin, embedded in paraffin, and processed into 6- μ m sections for hematoxylin-eosin staining to evaluate the mean thickness.

Assessment of Uniformity of the Amount of Air Leakage

Another 3 beagle dogs were used to evaluate the quantitative uniformity of air leakage from pleural defects. After a median sternotomy, both pleural cavities were opened, and 5 lesions per animal were created on 3 lobes in the right lung and 2 lobes in the left lung by the same surgeons using the same procedure. The amount of air leakage from the resected area was quantified using the collection over water technique. The outer case of a 50-mL syringe, the tip of which was connected to a tube, was pushed onto the lung surface so that the resected area was completely covered and any air leakage was shut in. The tip of the tube was inserted into a graduated cylinder in a tank filled with water at room temperature. The inhalation setting of the ventilator was changed to 100% oxygen to avoid any influence of nitrous oxide dissolution into the water. The central part of the irrelevant lung lobe was occluded with a soft tissue clamp to stop ventilation and air leakage. The amount of air leakage was quantified as the amount of gas collected in the graduated cylinder when the airway pressure was held at 15 cm/H₂O for 10 seconds (Figure 2).

Penetration of Cyanoacrylate

The influence of cyanoacrylate on the pleura and lung parenchyma was examined using 1 beagle dog with 3 mg of fluorescein-4-isothiocyanate (FITC) (Dojindo Laboratories, Kumamoto, Japan) dissolved in 1 mL of *n*-butyl cyanoacrylate just before surgery. After left thoracotomy, 5 pleural defects were created by the same method using the FITC-labeled cyanoacrylate. The resected tissues were frozen in Tissue-Tek optimal cutting temperature compound (Sakura Finetek USA, Inc, Torrance, Calif), and frozen sections (10 μ m) were serially cut with a cryostat. After drying at room temperature, the slides were rinsed with phosphate-buffered saline, followed by nuclear counterstaining with 4',6-diamidino-2-phenylindole (Sigma, St Louis, Mo). Penetration of FITC-labeled cyanoacrylate into the lung parenchyma was assessed by immunofluorescence microscopy.

Histologic Differences Arising From Three Resection Procedures

A long-term experiment was performed using 2 beagle dogs to observe the histologic differences in the lung parenchyma arising from conventional resection procedures. Ampicillin sodium at 50 mg/kg was administered intravenously just before the operation. After thoracotomy, 3 pleural defects were created in the upper lobe of the left lung. Each site was resected by 3 different procedures as follows. Procedure A: The pleura was resected using only a microtome blade, and hemostasis was obtained by sponge pressure. Procedure B: The pleura was resected using only a microtome blade, and hemostasis was obtained by minimum use of electrocautery (Coagulation 60 W, ACUTOR SK-2, Acoma Medical Industry Co, Ltd, Tokyo, Japan). Procedure C: The pleura was resected using cyanoacrylate and a microtome blade, and hemostasis was obtained by sponge pressure. Procedures A and B were conventional procedures,¹⁰⁻¹⁴ and procedure C was our procedure. The thoracic cavity was thoroughly lavaged, a 10F chest tube was placed, and the wound was closed in layers. The chest tube was removed after disappearance of air leakage. Thoracic radiographs were taken on the day after removal of the chest tube to evaluate the inflation of the lung. The animals were euthanized at 2 and 4 weeks after surgery for gross observation and to investigate the histologic changes after pleural resection. The resected lungs were reinflated with 10% formalin and immersed in the same solution. The fixed specimens were embedded in paraffin, sectioned, and subjected to hematoxylin-eosin, Masson trichrome, and elastic-Van Gieson staining.

Statistical Analysis

All values are presented as the mean $\pm$ standard deviation. Differences in the time required for hemostasis and the thickness of the resected tissues were analyzed for significance using the 1-factor analysis of variance.

Results

Morphologically, round areas of the pleura were uniformly resected with cyanoacrylate and microtome blades (Figure 3, A). Pulmonary air leakage was observed from the whole area of each lesion when the airway pressure was held at 15 cm/H₂O. Histologically, the resected tissue consisted of

Figure 3. Macroscopic view of the resected side and histologic examination of the resected tissues (hematoxylin–eosin stain, magnification $\times 400$). A, Morphologically, uniformly round areas of pleura were resected using our procedure. B, Histologically, the resected tissue consisted of pleura and thin underlying lung parenchyma (scale bar = 100 μm).

pleura and thin underlying lung parenchyma (Figure 3, B). The mean time required for hemostasis performed by the 3 respiratory surgeons was 1.7 ± 1.2 minutes, 1.6 ± 0.5 minutes, and 1.5 ± 0.7 minutes ($P = .69$), respectively, and the mean thickness of the resected tissue was $141 \pm 56 \mu\text{m}$, $155 \pm 34 \mu\text{m}$, and $114 \pm 37 \mu\text{m}$ ($P = .13$), respectively. The mean amount of air leakage from the resected area was $90 \pm 6 \text{ mL}$, $106 \pm 21 \text{ mL}$, and $109 \pm 16 \text{ mL}$ ($P = .19$),

TABLE 1. Results of pleural defect model created by our procedure among three surgeons

	Surgeon A	Surgeon B	Surgeon C	P value
Time required for hemostasis (min)	1.7 ± 1.2	1.6 ± 0.5	1.5 ± 0.7	.69
Thickness of resected tissue (μm)	141 ± 56	155 ± 34	114 ± 37	.13
Amount of air leakage (mL)	90 ± 6	106 ± 21	109 ± 16	.19

respectively. There were no significant differences in these data among the 3 surgeons (Table 1).

Immunofluorescence microscopy confirmed that the cyanoacrylate remained entirely on the surface of the pleura (Figure 4), and no penetration into the lung parenchyma was observed at any of the resection sites.

In the long-term experiment, all the dogs survived uneventfully and did not demonstrate any obvious complications. The chest tube was removed on the day after the operation in all cases. Chest radiographs showed inflation of the lung, and macroscopically there were no adhesions or infections in the resected areas. Histologic examination showed that in procedure A, the resected area was evident as a thick fibrocellular layer with mesothelial coverage. Infiltration of fibroblasts and lymphocytes into the alveolar interstitium was observed only in the surface layer of the lung parenchyma (Figure 5, A, B). In procedure B, the tissue response caused by thermal injury was severe. Destruction of the alveoli extended to the deep lung parenchyma with severe infiltration of fibroblasts. At 4 weeks, the destroyed layer had been replaced by severe fibrosis (Figure 5, C, D). In procedure C, the resected area was covered by a single layer of mesothelial cells and a thin fibrotic layer with underlying normal lung parenchyma (Figure 5, E, F).

Discussion

Prolonged postoperative pulmonary air leakage is one of the most troublesome complications of lung surgery. Fibrin glue is frequently applied as a sealant to prevent air leakage, and its effective hemostasis and air-sealing properties have been reported by many investigators.^{16–18} However, fibrin glue has a disadvantage in its industrial production, because human blood is used as its source. Furthermore, when biologic materials are used, complete prevention of infectious contam-

Figure 4. Immunofluorescence microscopy of the resected pleura (4',6-diamidino-2-phenylindole stain, magnification $\times 400$). FITC-labeled cyanoacrylate remained entirely on the surface of the pleura, and no penetration into the pleura and lung parenchyma was observed. FITC, fluorescein-4-isothiocyanate.

Figure 5. Histologic examination of the lung after resection of the pleura by 3 different procedures. (Masson trichrome stain, magnification $\times 100$). *A, B*, In procedure A, histologic changes were limited to the superficial layer of the lung parenchyma as a thick fibrocellular layer. *C*, In procedure B, the tissue response to thermal injury was severe. Destruction of the alveoli extended to the deep lung parenchyma. *D*, At 4 weeks, the destroyed layer was replaced by severe fibrosis. *E, F*, In procedure C, the resected area was covered by a thin fibrotic layer with normal underlying lung parenchyma.

ination is difficult. Therefore, new synthetic sealants have been developed and investigated using animal models.^{4,14}

An animal model with a uniform pleural defect is indispensable for evaluation of seal pressure and histologic changes after application of synthetic sealants. However, it is difficult to create pleural defects with uniform size and depth because of the characteristics of the lung, which has soft tissue, a round shape, and a smooth surface. To resect the pleura with only scalpels, scissors, or blades, the lung surface must be raised with forceps while the lung is inflated. Hemostasis at the resected area often takes a long time to achieve using sponge pressure alone because of the depth of the resulting defect. In our preliminary study, it took more than 10 minutes to achieve hemostasis by sponge pressure alone. Therefore, hemostasis often had to be done with minimal use of electrocautery. However, the resulting thermal injury caused various degrees of histologic change. For the precise evaluation of histotoxicity after application of synthetic sealants, histologic changes after pleural resection must be minimized as much as possible.

To create a uniform pleural defect in this study, we used *n*-butyl cyanoacrylate, which has already been used clinically for embolization of blood vessels^{19,20} and as a tissue adhesive.²¹ The hallmarks of cyanoacrylate are its powerful adhesive properties for tissues and instantaneous polymerization. On contact with biological tissues in a moist environment, cyanoacrylate rapidly polymerizes to create a thin elastic film with high tensile strength, which guarantees firm

adhesion of tissues. The glue begins to solidify within 1 to 2 seconds, and the process is complete within 60 to 90 seconds. Once solidification has occurred, the glue no longer possesses adhesive properties, so that tissues or surgical gauze may be safely used in the operative field with no risk of undesirable adhesion.²²

The soft lung surface must be made firm and formed into a regular shape for uniform and thin resection. Cyanoacrylate polymerized immediately after application, and a patch of the adhesive of the desired shape could be created using a silicone ring, because silicone shows no bonding with cyanoacrylate. The hardened bonding agent allowed easy horizontal sliding of a microtome blade, and the formation of a flat surface made it possible to resect the pleura with a uniform thickness. In this way, cyanoacrylate enabled us to resect a minimum thickness of the pleura with a regular shape. In addition, the very thin slice of the pleura obtained resulted in less bleeding.

Cyanoacrylate polymers yield formaldehyde as a by-product of hydrolytic degradation. The released formaldehyde is histotoxic and can cause acute and chronic inflammation.²³ To evaluate the histologic changes caused by synthetic sealants, any remnant cyanoacrylate in the lung must be avoided. Labeling of cyanoacrylate with FITC made it possible to evaluate the penetration of cyanoacrylate into the lung parenchyma. The quantity of FITC was slight and did not influence the adhesive strength and immediate polymerization of cyanoacrylate. Immunofluorescence mi-

croscopy confirmed that cyanoacrylate was anchored to the surface of the lung, without penetration into the lung parenchyma. Therefore, these results showed that cyanoacrylate had no influence on the lung parenchyma after resection of the pleura using our procedure.

To evaluate the influence of resection procedures on the lung parenchyma, we compared 3 different procedures, including 2 conventional methods. When hemostasis was obtained with the use of electrocautery, destruction of the alveoli and severe infiltration of fibroblasts into the lung parenchyma were observed as a result of thermal injury. The destroyed layer was replaced by severe fibrosis 4 weeks after the procedure, and this is inappropriate as a model for testing the efficacy of prospective synthetic sealants. Therefore, to create a pleural defect model for testing new synthetic sealants, hemostasis should be obtained without the use of electrocautery. When the pleura was resected without using cyanoacrylate, hemostasis was obtained using sponge pressure, and histologic changes were limited to the superficial layer of the lung parenchyma. This layer was identified as a thick fibrocellular layer with infiltration of fibroblasts and lymphocytes. These histologic reactions were mild and considered not to influence assessment of the histotoxicity of synthetic sealants to a significant degree. However, this conventional procedure had 3 other problems. First, it was difficult to resect areas of the pleura with a uniform size and shape. Second, hemostasis took a long time. Third, the appearance of air leakage after hemostasis was partial because of formation of a blood clot. More bleeding, application of pressure for a long time, and formation of a blood clot might influence the lung parenchyma. Therefore, a pleural defect should be created with less bleeding to overcome these problems. Our procedure using cyanoacrylate allowed the creation of pleural defects with a uniform shape and depth, which required only a short time for hemostasis, and resulted in a similar amount of air leakage from the resected area. There were no histologic changes in the lung parenchyma after pleural resection. Clinically, pleural defects with various degrees of depth have often arisen from segmentectomy, synechiotomy, or interlobar transection. The lesions created by our procedure are superficial and might not completely reflect clinical situations. The characteristics of our pleural defect model are uniformity and repeatability. Furthermore, quantification of air leakage showed our pleural defect model produces large air leakage. Therefore, our procedure would be useful for the evaluation of seal pressure and histotoxicity after application of synthetic sealants.

Conclusions

A beagle model with a uniform pleural defect of the lung was created by using a silicone ring, cyanoacrylate, and a microtome blade. Histologic assessment demonstrated that the resected tissue was composed of pleura and thin underlying lung parenchyma with a thickness of approximately

150 μm . Repeatability by 3 respiratory surgeons was acceptable in terms of the shape and thickness of the resected tissue, the time required for hemostasis, and the amount of air leakage from the resected area. Immunofluorescence microscopy showed no influence of cyanoacrylate on the lung parenchyma. There were no histologic changes in the lung parenchyma after resecting the pleura using our procedure, compared with conventional procedures. Our model for creating pleural defects may be feasible for the evaluation of newly developed synthetic sealants.

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