Electrocautery-Assisted Pleural Puncture for a Highly Reproducible Rat Pneumothorax Model

Atsushi SUGA[*1], Tomoki NAKAGAWA[*2], Hajime WATANABE[*2], Shota YAMAMOTO[*3], Shunsuke YAMADA[*2], Terumitsu HASEBE[*4] and Ryota MASUDA[*1]

[*1]*Division of General Thoracic Surgery, Department of Surgery, Tokai University School of Medicine*
[*2]*Division of General Thoracic Surgery, Tokai University Hachioji Hospital*
[*3]*Division of Allergy, Pulmonary and Critical Care Medicine, Department of Medicine, University of Wisconsin–Madison, Madison, WI, USA*
[*4]*Department of Radiology, Tokai University Hachioji Hospital, Tokai University School of Medicine*

(Received February 4, 2026; Accepted March 13, 2026)

Objective: To describe a simple and reproducible rat pneumothorax model that is suitable for future sealant testing.
Methods: Female Wistar rats (16 weeks) underwent left thoracotomy under mechanical ventilation. While the lung was inflated, the visceral pleural surface was cauterized using monopolar electrocautery with a ball electrode (coagulation mode, power 20%, 0.5 s), and then punctured with a 21G needle to a depth of 0.5 cm (Model E). The air-leak threshold airway pressure was measured using a water seal test. Pneumothorax and re-expansion were assessed by fluoroscopy, and pleural repair was evaluated histologically at 4 weeks.
Results: Pneumothorax was induced in all rats (12/12) without procedure-related mortality. The air-leak threshold pressure ranged from 12 to 25 cmH$_2$O (mean 17.3; median 16). All rats showed complete lung re-expansion at 1 week. Histological findings revealed fibrotic pleural repair (~100 μm thick) with preserved deep alveolar architecture.
Conclusion: Electrocautery-assisted pleural puncture provides a feasible and reproducible rat pneumothorax model for subsequent preclinical studies.

Key words: pneumothorax model, rat, air leak, pleural defect

INTRODUCTION

Biocompatible sealants and covering materials are widely used to manage intraoperative air leakage and postoperative pneumothorax when lung resection or suturing is challenging [1–3]. Preclinical development and evaluation of these materials require a small-animal model that produces a consistent pleural defect with measurable air leakage while allowing postoperative survival and predictable healing. Although several rat pneumothorax/air-leak models have been reported, pneumothorax induction can be inconsistent in practice, often due to bleeding and clot formation that rapidly seals the defect. In this brief communication, we describe a simple electrocautery-assisted pleural puncture technique that reliably induces pneumothorax in rats and analyze the air-leak threshold pressure, fluoroscopic course, and histological repair in this model.

MATERIALS AND METHODS

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 animal experiments were approved by the Institutional Animal Care and Use Committee of Tokai University School of Medicine (approval No. 222061) and were conducted in accordance with institutional guidelines. First, we verified the reproducibility and reliability of pneumothorax models using various procedures that cause air leaks, including previously published techniques (Table 1).

Experiments were performed on the left lungs of 10 female Wistar rats aged 16 weeks. We used procedures A through E (from Table 1) to create pleural defects in anesthetized rats, and mainly evaluated the incidence of pneumothorax and bleeding from the pleural defect. Since our original procedure E (electrocautery followed by pleural puncture) yielded the best results, we used this particular method in further experiments.

Subsequent experiments using electrocautery followed by pleural puncture were performed on the left lungs of 12 female Wistar rats aged 16 weeks. Details are as follows. 1) Anesthesia was induced with Sevoflurane inhalation. Tracheal intubation was conducted using the outer casing of a 14G 48 mm long indwelling venous needle (Angiocath™ BD, New Jersey, U.S.A.). Then, the procedure was performed under ventilatory management (tidal volume of 3 mL, respiratory rate of 60 breaths/min). 2) The animal was placed in a right lateral decubitus position, and a 2 cm skin incision was made on the left fifth intercostal

Tomoki NAKAGAWA, Division of General Thoracic Surgery Tokai University Hachioji Hospital, 1838 Ishikawamachi, Hachioji, Tokyo 192-0032, Japan
Tel: +81-42-639-1111 Fax: +81-42-639-1144 E-mail: lovehohono@gmail.com

Table 1 Preliminary Experiments.

Model	Procedure	Result	No. of animals with confirmed pneumothorax	No. of animals tested
A	Direct puncture with a 21G needle	Blood clots due to bleeding from puncture site	0	2
B	Circular excision (diameter: 5 mm)	Blood clots due to bleeding at the resection site	0	2
C	Puncture after cauterization of the 5 mm circular resection site	Pneumothorax confirmed, animal died of tension pneumothorax after chest closure	1	1
D	Puncture using a 21G needle heated with a gas lighter	Pneumothorax confirmed	1	2
E	Electrocautery followed by pleural puncture	Pneumothorax confirmed	3	3

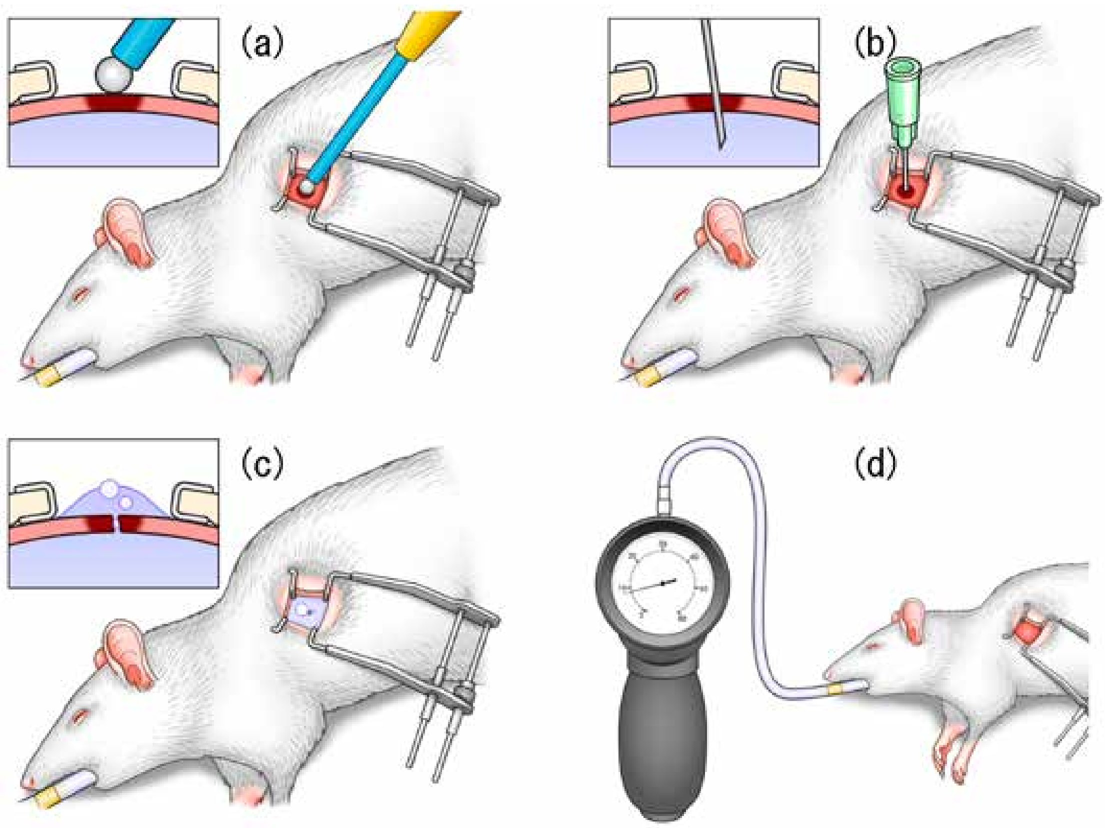

Fig. 1 Procedure for creating pneumothorax in Model E. An additional schematic of the cross-section is shown in the upper left corner. (a) While the lung was inflated, the pleural surface was cauterized with an electrocautery scalpel (monopolar scalpel connected to a ball-shaped electrode, power 20%, coagulation mode) for 0.5 s. (b) The cauterized site was punctured with a 21G needle to a depth of 0.5 cm. (c) After the thoracic cavity was filled with physiological saline solution, the water seal test was performed to confirm lung air leakage. We confirmed the airway pressure at which continuous air bubbles were observed by 2 researchers. (d) We used a cuff pressure gauge that can inflate the cuff.

space, after which the chest was opened. The lung surface was cauterized with an electrocautery scalpel (monopolar scalpel connected to a ball-shaped electrode with a diameter of 3 mm, coagulation mode at 20% power, Electrosurgical Unit SHAPPER mini®, MERA Co., Ltd. Tokyo, Japan) for 0.5 s to prevent bleeding during the next procedure while the lung was inflated (Fig. 1a). 3) The cauterized site was punctured (0.5 cm depth) using a 21G needle with a length of 16 mm (TERUMO, Tokyo, Japan) (Fig. 1b). 4) Warm saline was injected into the thoracic cavity, and mechanical ventilation was stopped. Then, positive pressure was manually applied to measure the airway pressure at which lung air leakage was observed (water seal test), using a cuff pressure gauge that can inflate the cuff (Sofit cuff inflator VS60VP®, MERA Co., Ltd. Tokyo, Japan) (Fig. 1c–d). 5) The chest and wound areas were then closed using sutures. 6) The animal was ventilat-

Fig. 2 Air-leak threshold airway pressure in the new pneumothorax model (Model E). Each dot represents an individual rat (n = 12). The horizontal line indicates the airway pressure at which continuous air leakage was first observed during the water seal test (range 12–25 cmH_2O, mean 17.3 cmH_2O, median 16 cmH_2O).

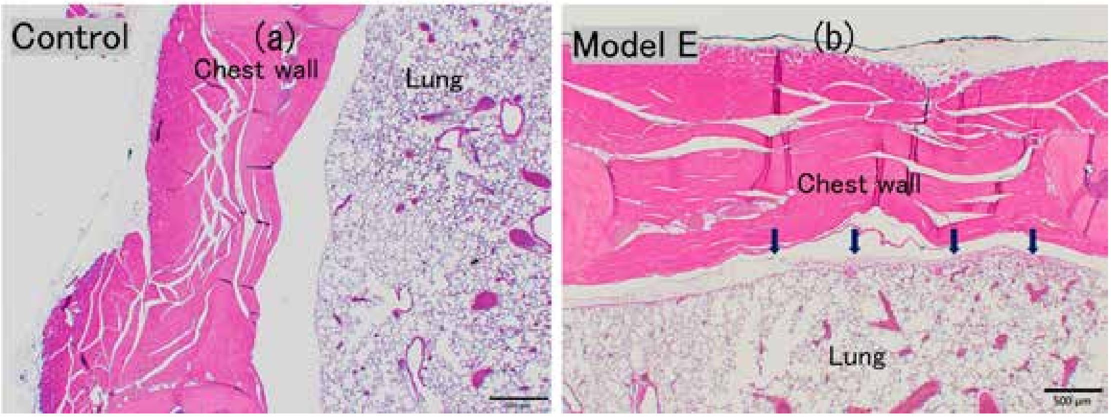

Fig. 3 Histological findings in lung tissue at 4 weeks after sham operation (a) and electrocautery-assisted pleural puncture (b). Fibrosis was observed on the lung surface at the ablation site (arrows). H&E stain; scale bar = 500 μm.

ed with positive pressure, and pneumothorax was confirmed under fluoroscopy. The condition was defined as mild pneumothorax if the pneumothorax was barely recognizable by fluoroscopy, severe pneumothorax if their lungs were completely collapsed, and moderate pneumothorax if they were at an intermediate stage. 7) The animals were weaned from mechanical ventilation in a state of moderate pneumothorax. Buprenorphine hydrochloride(0.05 mg/kg) was injected subcutaneously for analgesia at that time. A thoracic drainage tube was not used. 8) One week later, lung expansion was confirmed again by fluoroscopy. 9) Animals were sacrificed by exsanguination 4 weeks after the procedure. Their lungs were removed and fixed in 20% formalin, then sectioned and subjected to hematoxylin and eosin (H&E) staining for pathological examination of the pleural repair site.

RESULTS

Verification of various rat pneumothorax models

Table 1 shows the evaluation results for various pneumothorax models (A through D) that were previously reported. Direct pleural puncture with a 21G needle (A) and circular excision of the lung surface (B) did not cause pneumothorax because of blood clot coverage. Puncture after cauterization of the 5 mm circular resection site (C) led to air leakage, tension pneumothorax, and death. Pleural puncture using a 21G needle heated with a gas lighter (D) induced pneumothorax in only one of the two animals. On the other hand, our original procedure, electrocautery followed by pleural puncture (E), induced pneumothorax in all three animals.

Measurement of airway pressure and pathological observation of pleural repair in the new pneumothorax model

We proceeded with procedure E in an additional 12 rats. The airway pressure at which air leakage was first observed ranged from 12 to 25 cmH_2O (mean 17.3 cmH_2O; median 16 cmH_2O) (Fig. 2). One week after surgery, fluoroscopy confirmed complete re-expansion of the left lung without residual pneumothorax in all animals. All rats recovered from the surgical procedure and survived for at least 4 weeks. Figure 3b shows the histological findings 4 weeks after electrocautery-assisted pleural puncture. The pleural surface was covered by fibrotic tissue with an approximate thickness of

100 µm, while the deeper alveolar structures were preserved.

DISCUSSION

In this brief communication, we describe an electrocautery-assisted pleural puncture technique (Model E) that induced pneumothorax in all rats (12/12) without procedure-related mortality. The air-leak threshold airway pressure measured by the water seal test ranged from 12 to 25 cmH_2O (mean 17.3; median 16). Clinically, pneumothorax or air leak is detected within 25 cmH_2O at most [4]. Fluoroscopy confirmed complete lung re-expansion in all animals at 1 week. Histology at 4 weeks showed fibrotic pleural repair with an approximate thickness of 100 µm and preserved deep alveolar architecture.

Several rat pneumothorax or air-leak models have been reported, including open pneumothorax models without visceral pleural injury and models involving simple puncture or superficial resection [5–7]. In our preliminary attempts (Models A–D), direct puncture or surface excision often caused bleeding, and clot formation rapidly sealed the defect, resulting in inconsistent pneumothorax induction. Conversely, more extensive injury combined with cauterization could produce an excessive leak and fatal tension pneumothorax after chest closure. Brief electrocautery of the inflated lung surface before puncture coagulates superficial capillaries, minimizes bleeding, and enables a standardized puncture to reliably maintain an air leak.

This model provides a practical platform for subsequent preclinical screening of sealants or covering materials because it yields a measurable pressure threshold for air leakage and involves a predictable healing course. Nevertheless, technical success may depend on the electrocautery system and operator, and our fluoroscopic severity grading remains semi-quantitative. This model can be produced at low cost using common devices.

Future studies should standardize device settings and objective imaging criteria so that the model can be validated across operators and institutions.

Pitfalls and practical tips:

- Overcauterization or a large ablation area can create an excessive air leak and lead to tension pneumothorax after chest closure. Therefore, limit cautery time to 0.5 s and keep the cauterized area small and superficial.
- Insufficient puncture depth may result in little or no air leakage. Therefore, puncture to a consistent depth (0.5 cm) perpendicular to the pleural surface.
- Define the air-leak threshold consistently (e.g., airway pressure at which continuous bubbles are first observed during the water seal test) and record the pressure using the same device.
- After closure, confirm pneumothorax under fluoroscopy and monitor animals closely for respiratory distress. Always incorporate clear humane endpoints in the protocol.

ACKNOWLEDGMENTS

The authors thank the Life Science Support Center, Tokai University, for technical assistance.

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