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Original Article

Allogeneic fibroblast sheets prevent pulmonary air leaks caused by rat pleural defects without adhesion to the thoracic wall

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ABSTRACT

Introduction: Pulmonary air leak (PAL) is one of a complication of thoracic surgery and an unavoidable problem after lung resection or pleural adhesion detachment. Conventional procedures to close PAL by applying polyglycolic acid (PGA) sheets are prone to pleural adhesion. This study evaluated the ability of allogeneic fibroblast sheet transplantation to prevent PALs.
Methods: Rat skin fibroblasts were prepared from transgenic rats expressing green fluorescent protein (GFP) and cultured on temperature-responsive culture dishes to harvest fibroblast sheets. Allogeneic fibroblast sheets or PGA sheets were transplanted onto the pleural defects of the left lung in F344/NJcl-rnu/rnu (athymic rat), Slc:SD (SD), and BN/SsNSlc (BN) rats to assess PAL and adhesion prevention. Histological and immunological analyses were conducted to evaluate lung tissue of PALs transplanted with fibroblast or PGA sheets.
Results: Fibroblast sheets and PGA sheets closed pleural defects with PALs in all rat models. Adhesions were observed in most rat models transplanted with PGA sheets, but no adhesions were observed in rat models transplanted with fibroblast sheets. Immunostaining for HBME-1 indicated the regeneration of pleura by fibroblast sheet transplantation on the defects without adhesions after 2 weeks and 3 months of transplantation.
Conclusions: Similar to autologous fibroblast sheet transplantation, the transplantation of allogeneic fibroblast sheets prevented PALs and closed pleural defects without adhesion between the visceral and parietal pleura. Therefore, it can be concluded that allogeneic fibroblast sheets can be used as a ready-to-use sealant for preventing postoperative PALs.

1. Introduction

Hemorrhage and infection are prevalent postoperative complications, but pulmonary air leaks (PALs) are unique to thoracic surgery. Postoperative PALs are an unavoidable complication following lung resection or pleural adhesion detachment [1–3]. Operative procedures for lung cancer include wedge resection, segmentectomy, and lobectomy, with lobectomy being the standard. The results of the Japan Clinical Oncology Group (JCOG) 0802/West Japan Oncology Group (WJOG) 4607L trial show that the prognosis of small-sized peripheral non-small cell cancer remains the same after segmentectomy, indicating a possible increase in the use of segmentectomy as a standard surgical procedure in the future [4]. However, sublobar resections such as wedge resection and

Abbreviations: α-SMA, alpha-smooth muscle actin; CT, computed tomography; FGF-2, fibroblast growth factor 2; GFP, green fluorescent protein; HE, hematoxylin and eosin; PAL, pulmonary air leaks.

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segmentectomy increase the resected area of lung parenchyma, and therefore may result in increased postoperative PAL [5].

Conventional procedures for PAL closure, such as the use of polyglycolic acid (PGA) sheets, can lead to pleural adhesion, which may restrict lung movement and reduce postoperative pulmonary function, which further require manipulations such as adhesion removal in ipsilateral thoracic surgery, making the surgery more difficult [6,7]. We previously reported PAL prevention from pleural defects in animal models using transplanted autologous fibroblast sheets [8,9]. Interestingly, the transplanted fibroblast sheets were not associated with adhesion formation to the parietal pleura. Human fibroblast sheets were used to demonstrate whether PAL was prevented by the sheet transplantation on pleural defect in an immunocompromised rat model, indicating prevention of PAL [10]. Therefore, these results indicate that autologous fibroblast sheets may be ideal sealants to prevent PALs following surgical procedures that involve pleurectomy.

A clinical study was conducted to assess the safety of an autologous dermal fibroblast sheet transplantation for closing pleural defects with PALs [11,12]. Autologous fibroblast sheet fabrication took approximately four weeks per participant. Since the fabrication of autologous cell products must be conducted according to good manufacturing practice guidelines [13], autologous cell product fabrication is time-consuming and costly.

Allogeneic fibroblast sheet transplantation effectively prevented PALs from rat pulmonary defects, and the results of the in vivo imaging demonstrate that the transplanted allogeneic cells do not remain after 2 weeks of the transplantation [14]. These results indicate that allogeneic fibroblast sheet transplantation prevents early postoperative PALs, but whether the fibroblast sheets prevents PALs beyond 2 weeks after the transplantation remains unknown. Furthermore, the allogeneic fibroblasts transplanted onto the pleural defect are rejected by immune cells to induce inflammation, causing defect adhesion to the parietal pleura [15].

In this study, we utilized three models of PALs in F344/NJcl-rnu/rnu rats (athymic rat), Slc:SD (SD) rats, and BN/SsNSlc (BN) rats, which are assumed to demonstrate different immune responses to reject the transplanted allogeneic fibroblasts derived from SD rats, to assess the effectiveness of preventing PALs after 2 weeks and 3 months of allogeneic fibroblast sheet transplantation and the pleural adhesion frequency.

2. Methods

2.1. Ethical statement

All animal experiments were performed following the guidelines of the Ethics Committee for Animal Experimentation of Tokyo Women's Medical University and in compliance with the Legislation and Regulation on the use of animals in biological research with the ARRIVE guidelines. All experimental protocols were approved by the Ethics Committee for Animal Experimentation of Tokyo Women's Medical University. All animals were housed in individual cages with free access to food and water under a light/dark cycle of 12 h and maintained at constant room temperature and humidity. Animals were euthanized by exsanguination under 5 % isoflurane in accordance with the American Veterinary Medical Association (AVMA) euthanasia guidelines.

2.2. Culture of rat dermal fibroblasts

Explant culture and subsequent passages were performed in a culture medium consisting of Dulbecco's modified Eagle's medium (Sigma, St. Louis, Missouri, USA) supplemented with 10 % fetal bovine serum (Moregate Biotech, Queensland Australia), 200 μmol/L of ascorbic acid, and 100 ng/mL of basic fibroblast growth factor (Trafermin, Kaken Pharmaceutical Co., Ltd., Tokyo, Japan). Abdominal skins were excised from 1–3-day-old male outbred rats (Slc:SD; Sankyo Labo Service Corporation, INC., Tokyo, Japan) or transgenic rats (SD-Tg [CAG-EGFP]; Sankyo Labo Service Corporation, INC.) that express a green fluorescent protein (GFP). The excised skins were cut into 2–3 mm pieces, and the dermal side was placed on 60-mm diameter dishes and cultured for 7–10 days. The fibroblasts were isolated by explant culture from dermal tissues. Primary cultured cells were subsequently dissociated with 0.05 % trypsin–ethylenediaminetetraacetic acid (EDTA), seeded on temperature-responsive culture dishes (35 mm in diameter; UpCell, CellSeed Inc., Tokyo, Japan) at a density of 1.0×10^5 cells/cm^2, and cultured for 10 days at 37 °C.

2.3. Rat fibroblast sheet transplantation in PAL rat models

The rat dermal fibroblast cells cultured on the temperature-responsive surfaces were harvested by temperature reduction to 20 °C. The male athymic rats (F344/NJcl-rnu/rnu; CLEA Japan Inc.), SD rats, and BN (BN/SsNSlc; Sankyo Labo Service Corporation, INC.) rats were intubated with an endotracheal tube, and anesthesia was maintained using isoflurane inhalation. The rats were placed in a right lateral decubitus position, and a left-lateral thoracotomy in the 4th–5th intercostal space was performed (Fig. 2a and b). Left pleural defects were established by 4-mm diameter punch biopsies, and the presence of continuous air bubbles confirmed air leakages (Fig. 2c). Fibroblast sheets were transplanted on the pleural defects, and left-lateral wounds were closed after confirming the absence of PALs from the defects. No drugs, such as steroids or immunosuppressive agents, were administered in the perioperative period. The rat was euthanized by exsanguination under isoflurane anesthesia 2 weeks or 3 months later, and the left lung containing the transplanted sheet was resected for histological analyses.

2.4. Evaluations of PALs and adhesions

PALs were assessed by micro-computed tomography (CT) scan (R_mCT2, RIGAKU, Tokyo, Japan) before lung resections. Rethoracotomy was conducted from the diaphragm side, and adhesions were visually confirmed when visualizing the caudal side (Fig. 2f). A CT scan was performed after opening the chest to assess adhesion between the visceral and parietal pleura (Fig. 2k and l).

2.5. Histology and immunohistochemistry

Tissues were fixed with 10 % formalin and routinely processed into 10-mm-thick paraffin-embedded sections. Hematoxylin and eosin (HE) and Azan staining were conducted by conventional methods. De-paraffinized sections were treated with one of the following antibodies for immunohistochemistry: mouse monoclonal anti-vimentin (1:500, DakoCytomation, Glostrup, Denmark), mouse monoclonal anti-alpha-smooth muscle actin (1:200, DakoCytomation), mouse monoclonal anti-HBME-1 (1:200, DakoCytomation), or rabbit monoclonal anti-GFP (1:100 dilution; ab290; Abcam, Cambridge, UK).

2.6. Statistical analysis

JMP Pro version 17.0.0 (SAS Institute, Cary, NC, USA) was utilized for data analysis. Adhesion assessments were compared between groups with the Chi-squared test. A p-value of <0.05 was considered statistically significant.

3. Results

3.1. Rat dermal fibroblast sheet fabrication

Rat dermal fibroblasts were prepared by explant culturing of abdominal skins derived from 1–3-day-old SD rats that were serially cultured. The rat fibroblasts were treated with 0.05 % trypsin–EDTA to seed the cells on temperature-responsive culture dishes. The fibroblasts proliferated and became confluent on the dishes after 10 days of the culture (Fig. 1a). The sheets of dermal fibroblasts were harvested by reducing the temperature to 20 °C (Fig. 1b). Histological analyses of the fibroblast sheets were conducted, indicating that the fibroblast sheets consisted of multilayered cells with little collagenous fiber depositions (Fig. 1c and d). Immunochemical analysis of the fibroblast sheets revealed that most cells in the sheets expressed GFP and vimentin (Fig. 1e and f). Previous studies have demonstrated that ascorbic acid and its derivatives in a culture medium affect fibroblasts to induce the deposition of collagen fibers, by which dermis-like tissue consisting of collagen fibers and multilayered fibroblasts is formed in vitro [16–18]. Therefore, the multilayered cells in the sheet consisted of cultured rat dermal fibroblasts. These findings indicated that the transplantable fibroblast sheets were prepared by cultivating rat neonatal dermal fibroblasts on temperature-responsive cell culture dishes.

3.2. Allogeneic fibroblast sheet transplantation

The fibroblast or PGA sheets were transplanted in PAL rat models to assess whether allogeneic fibroblast sheets prevent air leaks. PALs from the pleural defects caused by partial resection of the left lung were prevented by fibroblast or PGA sheet transplantations without any procedures for closing the defect by suture or stapling (Fig. 2d, e, g, h). Green fluorescence was observed on the transplantation region immediately after GFP-fibroblast sheet transplantations onto the pleural defects in athymic rats (Fig. 2i). Athymic rats demonstrated GFP-positive cells derived from the GFP-fibroblast sheet after 2 weeks of the transplantation but not SD and BN rats (data not shown). The CT scan, after 2 weeks and 3 months of PGA or fibroblast sheet transplantations, revealed no evidence of collapsed lungs before re-thoracotomy (Fig. 2j).

3.3. Evaluation of pleural adhesions by allogeneic fibroblast sheet transplantation

Allogeneic fibroblast or PGA sheets transplanted onto the pleural defect were assessed for defect adhesion to the parietal pleura in athymic, SD, and BN rats, exhibiting different immune responses against allogeneic cell grafts. After 2 weeks and 3 months of the transplantation, re-thoracotomy was conducted from the diaphragm side, and the adhesions were visually confirmed by observing from the caudal side. Parietal pleural adhesions were observed by CT scan after 2 weeks of PGA sheet transplantation (Fig. 2k). No adhesions were observed by CT scan after 2 weeks of the fibroblast sheet transplantation (Fig.2l). Adhesions were formed in all cases of athymic and SD rats transplanted with the PGA sheets at 2 weeks and 3 months, but none with fibroblast sheets (Table 1). No adhesions were observed in 4 and 3 cases of BN rats transplanted with PGA sheets after 2 weeks and 3 months, respectively, but not at all with fibroblast sheets (Table 1). Significant differences between PGA and fibroblast sheet transplantations were observed in the athymic and SD rat PAL models at 2 weeks and 3 months (Table 1). The adhesions were not induced by allogeneic fibroblast sheet transplantation in all cases despite no significant difference between PGA and fibroblast sheet transplantations in the BN rat model (Table 1).

3.4. Histological analyses of rat PAL models

Histological and immunological analyses were conducted to assess the repair of pleural defects by allogeneic fibroblast sheet transplantation. PGA sheets were observed in all cases transplanted with PGA after 2 weeks, but not in all cases transplanted with PGA after 3 months (Fig. 3). Conversely, no differences were found in wound healing and collagen fiber deposition at the pleural defect in athymic, SD, and BN rats transplanted with allogeneic fibroblast sheets (Fig. 4). Immunostaining for GFP revealed the presence of positive areas in the fibroblast sheets in athymic rats but not in SD

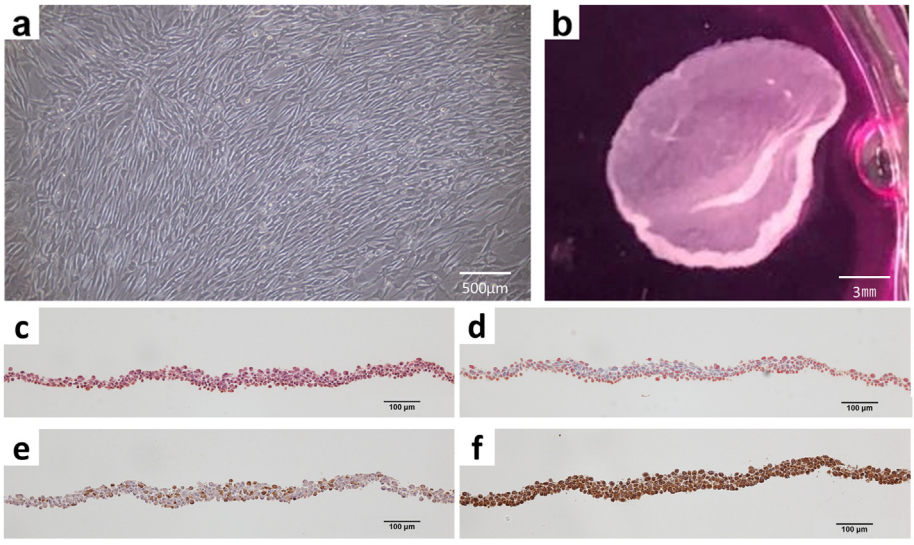

Fig. 1. Characterization of the neonatal Slc:SD (SD) rat dermal fibroblast sheet. (a) A phase-contrast micrograph of neonatal SD rat dermal fibroblasts cultured on a temperature-responsive culture dish for 10 days. (b) A macroscopic view of a dermal fibroblast sheet harvested by reducing the temperature to 20 °C. Hematoxylin and eosin (HE) (c) and Azan (d) staining demonstrate that the fibroblast sheets are composed of multilayered cells. Immunohistological analyses indicate green fluorescent protein (GFP) (e) and vimentin expressions (f). Scale bars indicate 100 μm.

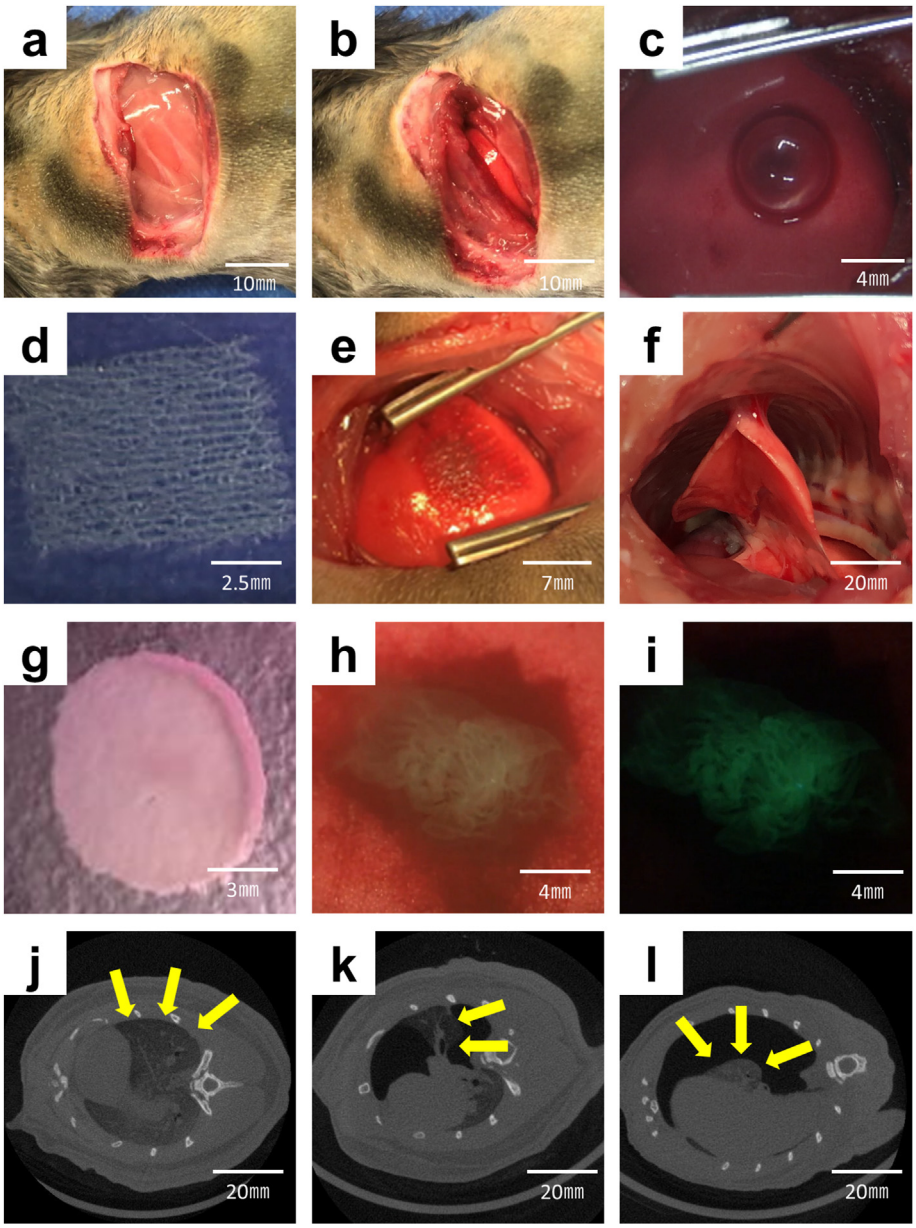

Fig. 2. Polyglycolic acid (PGA) or fibroblast sheet transplantations on a rat pulmonary air leak (PAL) models. (a) F344/NJcl-rnu/rnu rats (athymic rats), Slc:SD (SD) rats, and BN/SsNSlc (BN) rats, which were intubated and anesthetized with isoflurane, were placed in the right lateral decubitus position, and the left side of the chests was incised to expose down to the ribs. The image is shown in a BN rat. (b) A left-lateral thoracotomy in the 4th–5th intercostal space was performed in a BN rat. (c) The presence of air bubbles from the pleural defect confirmed PALs. (d) A PGA sheet cut out into 1 cm × 1 cm squares. (e) PGA sheets transplanted onto the pleural defects in a BN rat. (f) Re-thoracotomy was conducted from the diaphragm side for visually confirming adhesions by observation from the caudal side. Pleural defects adhered to the parietal pleura after 2 weeks of PGA sheet transplantation in a BN rat. (g) Macroscopic view of a skin fibroblast sheet harvested by reducing the temperature to 20 °C. (h) Immediately after the transplantation of fibroblast sheets onto the pleural defect in an athymic rat. (i) Green fluorescent protein (GFP) expression in the fibroblast sheet observed by LED light immediately after the transplantation in an athymic rat. (j) After 2 weeks of the transplantations of the fibroblast sheets, a computed tomography (CT) scan revealed no evidence of collapsed lungs before re-thoracotomy in an athymic rat. (k) Parietal pleural adhesions observed by CT scan after 2 weeks of PGA sheet transplantation in a SD rat. (l) No adhesions by CT scan after 2 weeks of the fibroblast sheet transplantation in an athymic rat.

and BN rats 2 weeks postoperatively; whereas no presence was observed in athymic, SD, and BN rats 3 months postoperatively. Normal lungs were also used for immunostaining for GFP as negative controls (Supplemental Fig. 1). Immunostaining for alpha-smooth muscle actin (α-SMA) revealed the presence of positive areas just below the PGA sheets after 2 weeks of transplantation (Fig. 5). Immunostaining for α-SMA demonstrated the presence of positive areas around the PGA and fibroblast sheets after 3 months of transplantation, probably due to neovascularization (Fig. 5). HBME-1 immunostaining, which is one of the mesothelial cell markers, was conducted to assess pleural defect regeneration. HBME-1-positve cells were observed on the regenerated pleural defects after 2 weeks and 3 months of fibroblast sheet transplantation although the immunostaining revealed no HBME-1-positive cells on adhesion tissue in the PGA transplantation group (Fig. 6).

4. Discussion

Previous studies indicated that autologous fibroblast sheet transplantation has demonstrated efficacy for preventing and

Table 1
The frequencies of pleural adhesion in pulmonary air leak models using F344/NJcl-rnu/rnu rats (athymic rat), Slc:SD (SD) rats, and BN/SsNSlc (BN) rats were evaluated after 2 weeks and 3 months of the transplantation of polyglycolic acid sheets (PGASs) or allogeneic dermal fibroblast sheets (FSs).

		2 weeks		3 months	
		adhesion	*P*	adhesion	*p*
Athymic rat	PGAS (N = 5)	5	0.002	5	0.002
	FS (N = 5)	0		0	
SD rat	PGAS (N = 5)	5	0.002	5	0.002
	FS (N = 5)	0		0	
BN rat	PGAS (N = 5)	1	0.292	2	0.114
	FS (N = 5)	0		0	

closing intra- and postoperative PALs in preclinical and clinical studies [8,9,11,12]. Autologous fibroblast sheet fabrication took approximately four weeks per participant in the clinical study. Therefore, in order to use PAL closure using dermal fibroblast sheet transplantation for pulmonary air leaks during emergency surgery or unexpected intraoperative air leaks, we evaluated PAL closure using allogeneic dermal fibroblast sheet transplantation in a rat model.

The fibroblast sheets derived from SD rats prevented PALs and closed the pleural defects in all cases despite using athymic, SD, and BN rats for PAL models. As the fibroblast sheets prevented PALs in both allogeneic SD rats and BN rats, they demonstrated the potential as an alternative to autologous fibroblast sheets for PAL prevention. Previous reports revealed that cryopreserved fibroblast sheets effectively closed pulmonary wounds with air leaks such as fresh fibroblast sheets [19–21]. Cryopreserved allogeneic fibroblast sheets may be utilized as ready-to-use sealants for preventing PALs in emergency surgery.

Intraoperative prevention of PALs is required for the survival of the rat model because pneumothorax and cardiac arrest occurred in a PAL rat model in which fibroblast sheets were not transplanted [8]. Loss of the allogeneic fibroblast sheet by the rejection may cause postoperative PAL and induce cardiac arrest due to tension pneumothorax because allogeneic cells are subject to immune rejection. The immunostaining for GFP indicated the presence of GFP-positive cells on the pleural defects in athymic rats after 2 weeks of transplantation, but no GFP-positive cells were found in SD and BN rats, in which the allogenic fibroblast sheets had disappeared. PAL was prevented without adverse events due to long-term transplantation after 3 months of allogeneic fibroblast sheet transplantation, despite the loss of the transplanted fibroblasts in SD and BN rats.

Postoperative PALs close within a few days; thus, fibroblasts derived from the allogeneic cell sheet may not be required to present for the long term after transplantation [14]. The product using allogeneic cells may reduce the risk because the long-term persistence of cells may increase the risk of unexpected complications caused by cultured cells in cellular products. Therefore, allogeneic fibroblast sheets can be used as a sealant for covering PAL with a better safety profile compared to autologous fibroblast sheets.

PGA sheets were utilized as a control group in this study since PGA sheets have been conventionally used for PALs. PGA undergoes non-enzymatic hydrolysis *in vivo*, and glycolic acid and lactic acid derived from PGA degradation increase tissue acidity and are metabolized. The pleural adhesion caused by thoracotomy with the PGA application for in vivo preventing air leak may be a prolonged inflammatory response due to the stimulation by acidification during the PGA degradation process [22]. Additionally, the inflammation prevents mesothelial cell migration, of which the pleura consists, onto the pleural defect, causing parietal pleural adhesion [15]. The lung parenchyma caused by the lung resection

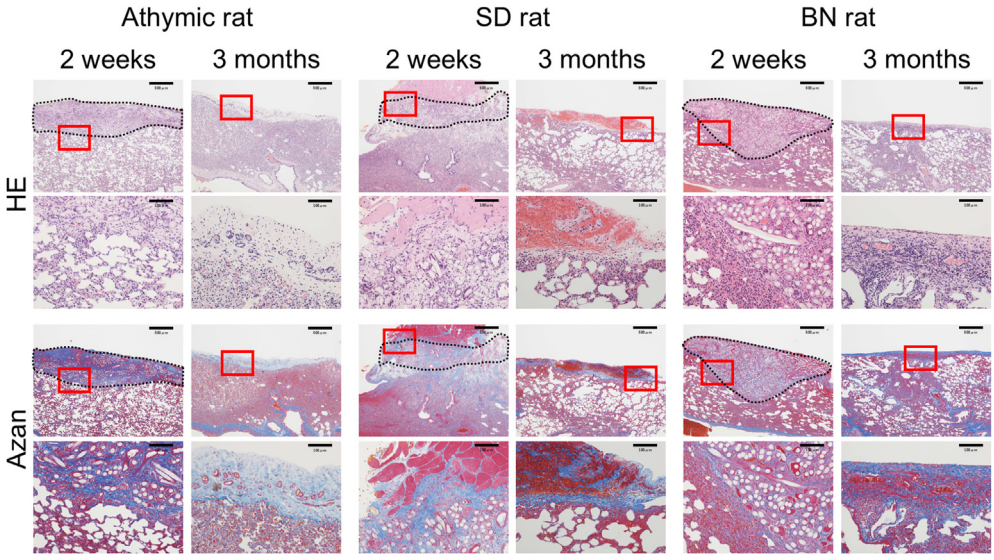

Fig. 3. Histological analyses for the pulmonary air leak (PAL) rat models after 2 weeks and 3 months of polyglycolic acid (PGA) sheet transplantation. F344/NJcl-rnu/rnu rats (athymic rat), Slc:SD (SD) rats, and BN/SsNSlc (BN) rats were utilized for the PAL models. The upper and lower panels represent hematoxylin and eosin (HE) staining and azan staining, respectively. The black dotted lines indicated the PGA sheet remaining after 2 weeks of the transplantation. Scale bars in the upper rows of each panel indicate 500 μm. Scale bars in the lower rows of each panel indicate 100 μm.

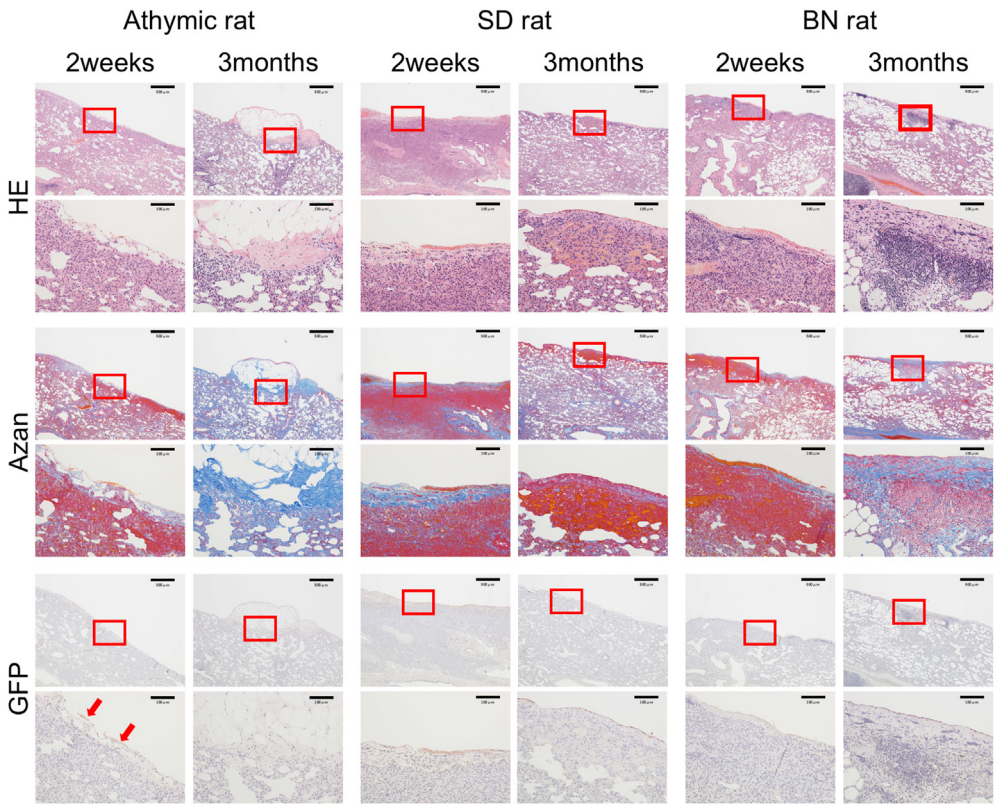

Fig. 4. Histological and immunochemical analyses for the rat pulmonary air leak (PAL) models after 2 weeks and 3 months of allogeneic dermal fibroblast sheet transplantations. F344/NJcl-rnu/rnu rats (athymic rat), Slc:SD (SD) rats, and BN/SsNSlc (BN) rats were utilized for PAL models. The upper, middle, and lower panels represent hematoxylin and eosin (HE), azan, and immunochemical staining of green fluorescent protein (GFP), respectively. Red arrows indicate stained areas for GFP. Scale bars in the upper rows of each panel indicate 500 μm. Scale bars in the lower rows of each panel indicate 100 μm.

that is absorbed by the sheet will induce further parietal pleural adhesion because PGA sheets are made of a felt-like material of PGA without hemostatic action [15,22–24]. The present study indicated that adhesions occurred at the pleural defects covered with PGA sheets. Conversely, no pleural adhesions were formed in all cases in which a fibroblast sheet was transplanted. The prevention of adhesions between the visceral and parietal pleura is crucial in maintaining lung function after thoracotomy for the quality of life

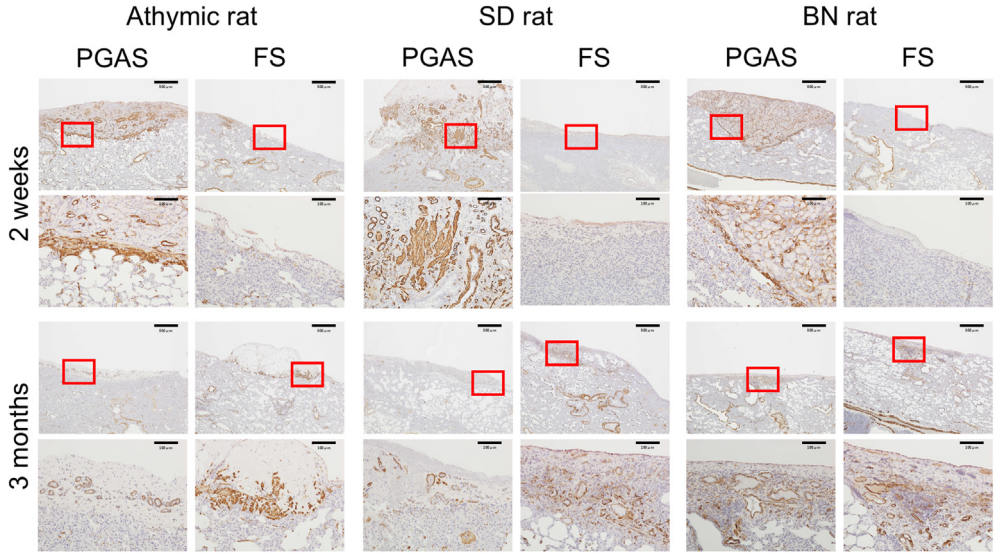

Fig. 5. Immunochemical staining of alpha-smooth muscle actin (α-SMA) for rat pulmonary air leak (PAL) models after 2 weeks and 3 months of polyglycolic acid (PGA) or allogeneic dermal fibroblast sheet transplantations. F344/NJcl-rnu/rnu rats (athymic rat), Slc:SD (SD) rats, and BN/SsNSlc (BN) rats were utilized for PAL models. The upper and lower panels represent the immunochemical staining of α-SMA after 2 weeks and 3 months of transplantations, respectively. Scale bars in the upper rows of each panel indicate 500 μm. Scale bars in the lower rows of each panel denote 100 μm.

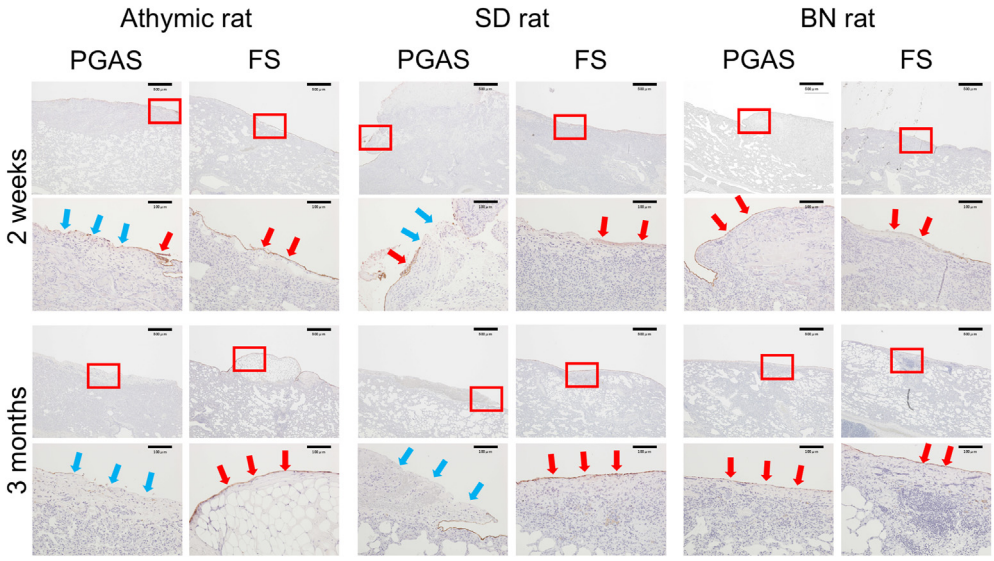

Fig. 6. Immunochemical staining of HBME-1 for rat pulmonary air leak (PAL) models after 2 weeks and 3 months of polyglycolic acid (PGA) or allogeneic dermal fibroblast sheet transplantations. F344/NJcl-rnu/rnu rats (athymic rat), Slc:SD (SD) rats, and BN/SsNSlc (BN) rats were utilized for PAL models. The upper and lower panels indicate the immunochemical staining of HBME-1 after 2 weeks and 3 months of transplantations, respectively. Red and blue arrows indicate stained areas for HBME-1 repaired on the pleural defects and not stained areas, respectively. Scale bars in the upper rows of each panel indicate 500 μm. Scale bars in the lower rows of each panel denote 100 μm.

of the patients [25]. Therefore, allogeneic fibroblast sheets that close PALs without adhesion would be useful sealants for thoracotomy.

In the present study, there are two limitations for investigating mechanisms to close a pleural defect by an allogenic fibroblast sheet without pleural adhesion. Although we hypothesized that pleural adhesions would be induced by PGA sheets in BN rats as in SD and athymic rats, the results of the experiments contradicted our hypothesis. The pleural defect transplanted with PGA sheets in some BN rats was not adhesions, probably because the pleura regenerated more rapidly than in athymic and SD rats, as shown by the immunostaining results for HBME-1. In the present study, we revealed that pleural adhesions were less likely to occur in BN rats for the first time. However, there is a possibility that the difference in the immune response in BN rats from athymic and SD rats accelerated the rapid regeneration of the pleura in BN rats. Therefore, further studies are required to clarify the relevance between the mechanism of rapid pleural regeneration and immune response in BN rats.

BN rats are widely used as model animals related to immune responses such as allergy and allogeneic transplantation, and we used BN rats as recipients of allogeneic cells in this study. In general, if BN rats, which carry RT1n haplotype [26], were used as recipients, inbred strain rats with different haplotypes of MHC from BN rats should be used as donors. Since outbred SD rats were used in this study, the diversity of MHC haplotypes is one of the concerns of an allogenic model. On the other hand, because the strain of available GFP transgenic rats were SD rats, the fibroblast cell sheets in SD rats were used for labeling and visualizing transplanted fibroblasts into recipient rats in this study. Although transplantation experiments should be performed with fibroblast sheets derived from other inbred rats, the use of GFP transgenic fibroblasts allowed us to show that the air leak could be occluded without adhesion even if allogeneic fibroblasts were lost after transplantation. However, future studies are required to evaluate whether allogeneic fibroblast sheet transplantation prevents pulmonary air leakage without pleural adhesion due to pleural defects using two different inbred strains of rats as donors and recipients.

5. Conclusion

Allogeneic fibroblast sheets were transplanted in PAL rat models to assess the prevention and closure of the pleural defect. The pleural defects were closed without PALs despite the loss of allogeneic fibroblasts derived from the transplantation in SD and BN rats. Moreover, allogeneic fibroblast sheet transplantation promoted pleural regeneration with a mesothelial cell layer, preventing adhesions between the visceral and parietal pleura. In conclusion, allogeneic fibroblast sheets may be effective as ready-to-use sealants for preventing PALs and closing pleural defects and emerge as an alternative to PGA and autologous fibroblast sheets. As a limitation, cryopreserved allogeneic fibroblast sheets are not used to evaluate the prevention of PALs, and the evaluation is necessary to use allogeneic fibroblast sheets as a ready-to-use sealant.

Declaration of competing interest

Tokyo Women's Medical University received research funding from CellSeed Inc. Tatsuya Shimizu is a shareholder of CellSeed Inc.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.reth.2025.01.012.

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