

A synthetic bioabsorbable sheet may prevent postoperative intrapleural adhesions following thoracotomy: a canine model

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Abstract

OBJECTIVES: Intrapleural adhesions following thoracotomy may be associated with prolonged operating time or a higher complication rate at reoperation. The aim of this experimental study was to investigate the anti-adhesion property of a bioabsorbable sheet following thoracotomy in a canine model.

METHODS: Ten adult beagle dogs underwent bilateral muscle-sparing thoracotomies with single ribs resected under general anaesthesia. A bioabsorbable sheet composed of poly-L-lactide copolymer (45 wt%) and ϵ -caprolactone (45 wt%) layered with polyglycolic acid (10 wt%) was sutured intrapleurally on the parietal pleura to cover the defect on the left, but not placed on the right side as a control. All the dogs were followed up with chest computed tomography until being sacrificed (6 months at the maximum). Thoracoscopic evaluations were performed at 1, 3 and 6 months for intrapleural adhesions at the thoracotomy site and absorption of the bioabsorbable sheet. The incidences of intrapleural adhesions were compared between the experimental side and the control side by the χ^2 test. Histological (macroscopic and microscopic) analyses of regenerated chest wall tissue were also performed at 1, 3 and 6 months.

RESULTS: All the dogs survived uneventfully until being sacrificed without any postoperative complications or significant radiological findings. The bioabsorbable sheet prevented intrapleural adhesions in all subjects. There were statistically significant differences in the incidence of intrapleural adhesions between the experimental side and the control side at the thoracotomy incision (0 vs 80%, $P = 0.0014$) at 1 month, (0 vs 66.7%, $P = 0.014$) at 3 months and (0 vs 75%, $P = 0.028$) at 6 months. The bioabsorbable sheet was found residual at 1, 3 and 6 months in all subjects. Histological analyses confirmed regenerated chest wall layers with significantly more capillary vessels at 1 month ($P = 0.015$), but not at 3 and 6 months ($P = 0.84$ and 0.41 , respectively), in the regenerated mucosal and submucosal layers on the experimental side.

CONCLUSIONS: Our findings suggest that the bioabsorbable sheet may prevent intrapleural adhesions with parietal pleurae regenerated with more vascularization at 1 month following thoracotomy. No adverse findings were noted with the sheet.

Keywords: Thoracotomy • Adhesion • Reoperation

INTRODUCTION

In general, any surgical wound can form postoperative adhesions that might cause postoperative complications or make reoperative procedures challenging [1–3]. To prevent laparotomy wounds from forming postoperative adhesions, anti-adhesive bioabsorbable materials have been developed [4, 5] and applied successfully to clinical practice for laparotomy wounds [6]. As for the thoracotomy wound, no antiadhesive material has been applied to clinical use although reoperative thoracic procedures, for example, for repeat pulmonary metastases or a second metachronous non-small-cell lung cancer, are not uncommon [7–9], and reoperative

thoracic procedures appear to carry a relatively high rate of postoperative complications [9–11]. The antiadhesive properties of several bioabsorbable materials have been validated only in murine thoracotomy models [12–16], which failed to translate into a large animal model [17, 18]. Therefore, in this experimental study, we aimed to investigate whether a synthetic bioabsorbable sheet, which has been applied to clinical practice as a dural substitute [19] following a large animal experiment [20], may prevent intrapleural adhesions in a canine thoracotomy model. In this experiment, we used a synthetic bioabsorbable sheet composed of poly-L-lactide copolymer, ϵ -caprolactone and polyglycolic acid; all of which were confirmed to be biocompatible and bioabsorbable [21, 22].

MATERIALS AND METHODS

A synthetic bioabsorbable sheet (Seamdura®)

Seamdura® (Gunze Limited, Ayabe, Japan) is a synthetic bioabsorbable dural substitute that has been indicated for the dural defect. Its components are poly-L-lactide copolymer (45 wt%) and ϵ -caprolactone (45 wt%) layered with polyglycolic acid (10 wt%) felt, which gives strength, elasticity and a leak-resistant seal. The thickness is ~ 220 μm . The sheet was sterilized with ethylene oxide gas and packed in a sterile manner. Seamdura® is degraded gradually by hydrolysis, and finally absorbed by the human body in ~ 8 months if placed intracranially. The strength of the sheet decreases by $\sim 50\%$ in 3–4 weeks following intracranial placement, and to nearly zero in 12 weeks.

Surgical procedures

Ten adult beagle dogs, weighing 8–14 kg each, were anaesthetized with the intramuscular administration of 15 mg/kg ketamine hydrochloride and 7 mg/kg xylazine and then intubated with a single-lumen endotracheal tube. Mechanical ventilation was maintained with sevoflurane. Prophylactic intramuscular ampicillin was administered. A left lateral, muscle-sparing thoracotomy was performed and a portion of the sixth rib was resected (Fig. 1A). The sterile 5×10 cm bioabsorbable sheet (Seamdura®) was brought to the surgical field (Fig. 1B). Using three No. 0 interrupted polydioxanone (PDS II®, Ethicon, Somerville, NJ, USA)

stitches around the upper and lower ribs (Fig. 1C), the sheet was placed and sutured intrapleurally (Fig. 1D and E) to cover the parietal pleural defect. All knots were placed extrapleurally (Fig. 1F). The left lung was fully expanded manually. No chest tube was placed and the wound was closed in layers. Then a right lateral, muscle-sparing thoracotomy was performed and the sixth rib was resected. As a control, no material was placed intrapleurally and the thoracotomy wound was closed with three No. 0 polydioxanone (PDS II®) pericostal stitches. The right lung was fully inflated manually. No chest tube was placed, and the wound was closed in layers. All the dogs received regular care postoperatively in the same way as preoperatively. All the dogs were followed up until being sacrificed (6 months at the maximum). The flow of postoperative evaluations is shown in Fig. 2.

All the surgical procedures were performed by Japanese board-certified thoracic surgeons (M.H. and F.K.) in accordance with the 'Guide for the Care and Use of Laboratory Animals' published by the National Institute of Health (NIH Publication No. 85-23, revised 1985). The experimental protocol was approved by Animal Experimental Committee of Kyoto University.

Postoperative follow-up with radiological evaluations (chest computed tomography)

Chest computed tomography (CT) was performed without contrast medium to evaluate the pleural space, chest wall, visceral pleura and lung parenchyma at 1, 3 and 6 months following thoracotomy. The images were obtained with a 16-row multi-detector

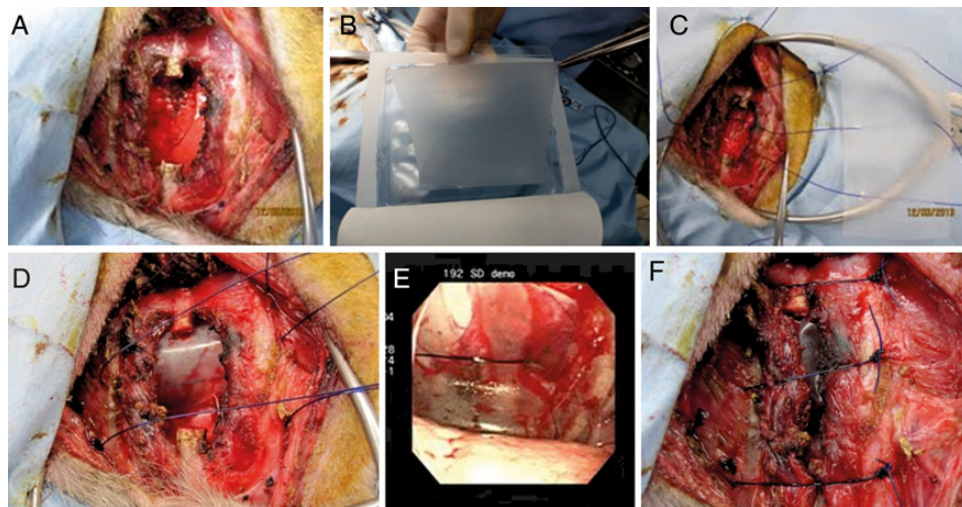


Figure 1: The procedural steps in the intrapleural placement (suturing) of the bioabsorbable sheet (Seamdura®). (A) A portion of the sixth rib was resected following a muscle-sparing lateral thoracotomy. (B) The sterile 5×10 cm bioabsorbable sheet (Seamdura®) was brought to the surgical field. (C and D) Using three No. 0 interrupted polydioxanone (PDS II®) stitches around the upper and lower ribs, the sheet was sutured intrapleurally. Before stitches were tied, the sheet stayed loose. (E) Thoroscopic view of the sheet sutured to the parietal pleura. (F) All knots were tied and the sheet was intrapleurally placed tight against the parietal pleura.

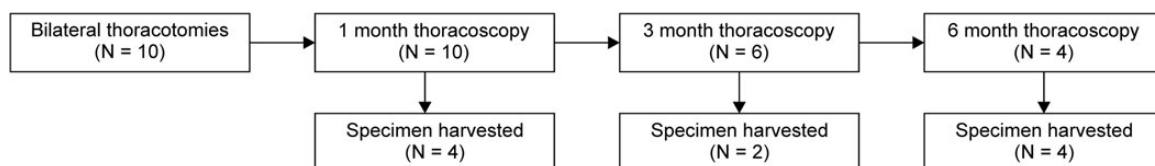


Figure 2: The flow of canine subjects following bilateral thoracotomies in this study.

CT scanner (Alexion 16, Toshiba Medical Systems, Tochigi, Japan) in the helical mode with 120 kV voltage, 50 mA per section, a 512 × 512 matrix and 7-mm slice thickness.

Thoracoscopic evaluation of intrapleural adhesions at thoracotomy, potential pleural defects at thoracotomy, and absorption of the sheet

At 1 month ($n = 10$), 3 months ($n = 6$) and 6 months ($n = 4$) following intrapleural placement (suturing) of the bioabsorbable sheet, exploratory thoracoscopy (Olympus®) through a 2-cm mid axillary incision on the eighth intercostal space was performed under general anaesthesia and double-lung ventilation via an endotracheal tube in the same way as mentioned above. Exploratory thoracoscopy was performed to evaluate intrapleural adhesions at the thoracotomy site bilaterally, potential pleural defects at the thoracotomy bilaterally, and absorption of the sheet on the experimental side. The lungs were inflated manually at the end of thoracoscopy.

Histological analysis of the regenerated chest wall tissue

The subjects were humanely killed with an intravenous injection of an overdose of sodium pentobarbital. A specimen was harvested from the regenerated chest wall tissue including parietal pleurae abutting the bioabsorbable sheet at 1 month ($n = 4$), at 3 months ($n = 2$) and at 6 months ($n = 4$). Macroscopic evaluations of all specimens were performed and then these were fixed in 10% phosphate-buffered formalin for at least 7 days, embedded in paraffin, and sectioned at 5 μ m. The regenerated chest wall tissues were sent for histological analysis by light microscopy after staining with haematoxylin and eosin. All specimens were examined by a board-certified pathologist (T.T.).

The number of capillary vessels in the regenerated mucosal and submucosal layers was counted in three randomly selected 10-power fields of view for each specimen. The number of capillary vessels was compared between the control side and the experimental side.

Statistical analysis

For comparison of the incidences of intrapleural adhesions following thoracotomy, the χ^2 test was used. For comparison of the number of capillary vessels, Student's *t*-test was used. All statistical tests were two-sided, and a *P*-value of <0.05 was defined as statistically significant. The JMP version 10.0.1 software (SAS Institute, Cary, NC, USA) was used for all statistical analyses.

RESULTS

Clinical course and radiological findings

All dogs survived uneventfully without any postoperative complications until being sacrificed. Chest CT did not show any pleural fluid or fluid collection above the chest wall or any significant change in visceral pleura or lung parenchyma at 1, 3 and 6 months (Fig. 3). The lungs were fully expanded without pneumothoraces.

Thoracoscopic evaluation of intrapleural adhesions at the thoracotomy incision and the bioabsorbable sheet

Intrapleural adhesions at the thoracotomy site are reported in Table 1, comparing the control side and the experimental side

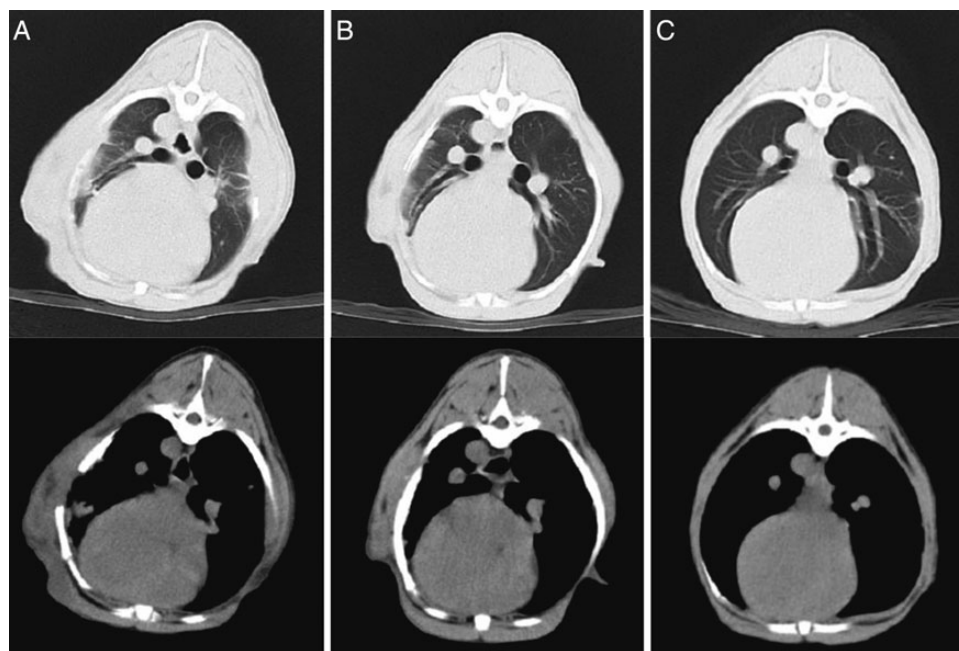


Figure 3: Representative images (upper images: lung window, lower images: mediastinal window) are shown from the follow-up chest computed tomography at 1, 3 and 6 months following placement of the bioabsorbable sheet. The bioabsorbable sheets were not identified due to radiolucency. No pleural fluid, seroma or significant finding in visceral pleurae and lung parenchyma was noted bilaterally at 1 month (A), 3 months (B) and 6 months (C).

where the bioabsorbable sheet was placed. Exploratory thoracoscopy at 1 month, while showing moderate-to-severe intrapleural adhesions at the thoracotomy site on the control side in 80% of the subjects (Fig. 4A), showed no intrapleural adhesions on the experimental side in all subjects (Fig. 4B). There was a statistically significant difference in the incidence of the intrapleural adhesions (0 vs 80%, $P = 0.0014$, relative risk: 0.000, 95% confidence interval (CI): 0.000–0.275) at 1 month. Significant differences were also noted at 3 months (0 vs 66.7%, $P = 0.014$, relative risk: 0.000, 95% CI: 0.000–0.648) and at 6 months (0 vs 75%, $P = 0.028$, relative risk: 0.000, 95% CI: 0.000–0.803). No additional adhesion developed at 3 months (Fig. 4E) or 6 months (Fig. 4F), and no adhesion regressed spontaneously (Fig. 4B and C). No pleural fluid was found at thoracoscopy, which was consistent with radiological findings. No

intrapleural adhesions were noted at the small incisions for thoracoscopy.

Bioabsorption of the sheet is summarized in Table 2 and no sheet was completely absorbed in 6 months.

Histological evaluation of regenerated chest wall tissue at the thoracotomy incision

Macroscopic findings of intrapleural adhesions and absorption of the sheet were compatible with thoracoscopic findings, as shown in Fig. 5.

The sections of regenerated chest wall tissue were compared between the control side and the experimental side at 1, 3 and 6 months (haematoxylin and eosin stain; original magnification $\times 10$), as shown in Fig. 6. Regenerated mesothelial layers, no granulation tissue and regenerated chest wall layers were observed in all specimens. At 1 month, the number of capillary vessels in the regenerated mucosal and submucosal layers was 12.1 ± 8.4 on the control side and 28.9 ± 9.7 on the experimental side in an average 10-power field of view ($P = 0.0013$). At 3 months, the number of capillary vessels in the regenerated mucosal and submucosal layers was 26.7 ± 4.0 on the control side and 24.5 ± 12.5 on the experimental side in an average 10-power field of view ($P = 0.84$). At 6 months, the number of capillary vessels in the regenerated mucosal and submucosal layers was 8.7 ± 1.5 on the control side and 10.0 ± 2.0 on the experimental side in an average 10-power field of view ($P = 0.41$). The mucosal and submucosal layers appeared thicker at 1 and 3 months than at 6 months on both sides, although no statistical analysis was performed.

DISCUSSION

Postoperative adhesions related to surgical wounds or intracorporeal procedures, varying in severity, may be associated with postoperative complications such as small bowel obstruction, infertility or chronic pain following laparotomy [1, 3], or more complications at reoperation [2]. A bioabsorbable sheet appears to

Table 1: Intrapleural adhesion at the thoracotomy site at 1, 3 and 6 months. Comparison of the control side (the right side) with the experimental side (the left side), where the bioabsorbable sheet (Seamdura®) was placed.

	(+) Intrapleural adhesion	(-) Intrapleural adhesion
At 1 month ($n = 10$)		
Control side (the right side)	8	2
Experimental side (the left side)	0	10
At 3 months ($n = 6$)		
Control side (the right side)	4	2
Experimental side (the left side)	0	6
At 6 months ($n = 4$)		
Control side (the right side)	3	1
Experimental side (the left side)	0	4

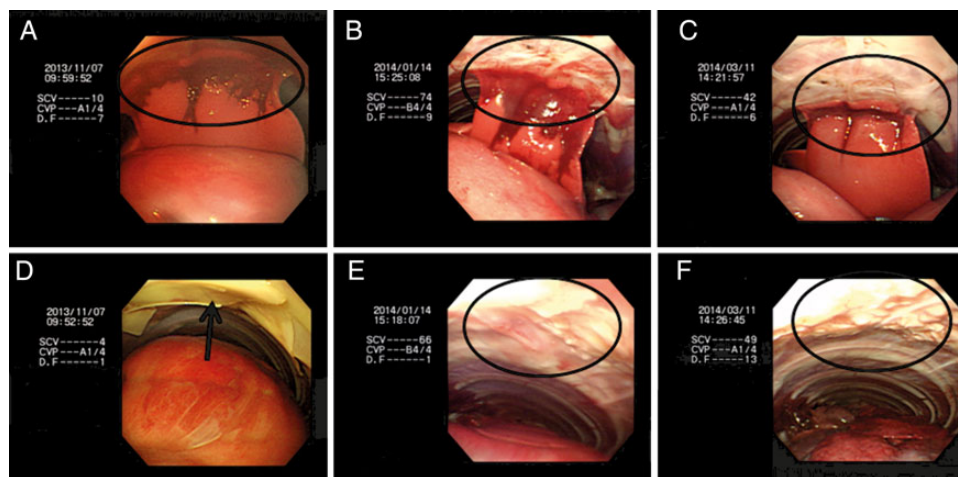


Figure 4: Representative images of exploratory thoracoscopy at 1, 3 and 6 months. An intrapleural adhesion was noted (encircled) at the thoracotomy site on the control side at 1 month (A), 3 months (B) and 6 months (C). On the other hand, no intrapleural adhesion was noted on the experimental side at 1 month (D), 3 months (E) and 6 months (F). The bioabsorbable sheet appeared almost intact (indicated by an arrow) at 1 month (d), and barely residual (encircled) on the parietal pleura at 3 months (e) and 6 months (f).

play a significant role in preventing adhesions following laparotomy, which is related to lower rates of postoperative complications [6] and potentially safer dissections at the time of reoperative procedures.

There is no material in clinical use to prevent intrapleural adhesions following thoracotomy in spite of the fact that pulmonary

resections on reoperated thoraces [7–9] are not uncommon. In an effort to develop anti adhesive materials for thoracotomy wounds, experimental studies in murine models have identified potentially effective bioabsorbable anti adhesive materials [12–16].

Of note, the previous experimental settings varied in the animal model (murine vs a large animal), the bioabsorbable material (biological vs synthetic or powder-type vs sheet-type) and the site of placement (on the visceral pleura vs on the parietal pleura).

We chose a large animal for this study, rather than murine models. First, anti adhesive materials for thoracotomy wounds have been validated only in murine models, and the findings of adhesion following thoracotomy in murine models were not compatible with those in canine models [17, 18]. Secondly, although there is no scientific background that suggests that the thoracic physiology of large animals, rather than murine models, resembles human thoracic physiology, surgical procedures (thoracotomy, wound closure, etc.) are more easily mimicked in large animal models.

Table 2: The absorption process of the sheet: macroscopic findings via exploratory thoracoscopy

	1 month	3 months	6 months
Almost intact	5/10 (50%)	0/6	0/4
>50% residual	3/10 (30%)	0/6	0/4
≤50% residual	2/10 (20%)	6/6 (100%)	4/4 (100%)
Completely absorbed	0	0	0

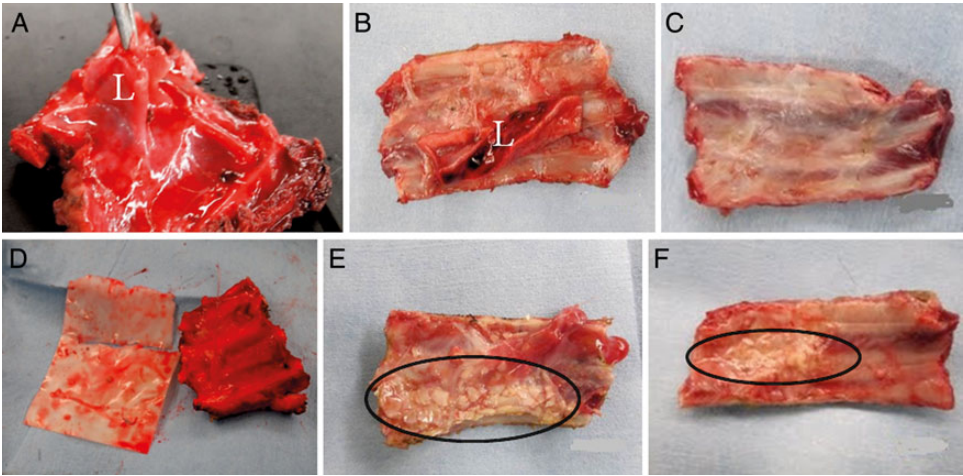


Figure 5: Representative macroscopic images of the specimens at 1, 3 and 6 months. The parietal pleura appeared to have regenerated on the control side at 1 month (A), 3 months (B) and 6 months (C) as well as on the experimental side at 1 month (D), 3 months (E) and 6 months (F). The bioabsorbable sheet appeared almost intact at 1 month (d), and barely residual (encircled) on the parietal pleura at 3 months (e) and 6 months (f). L: lung.

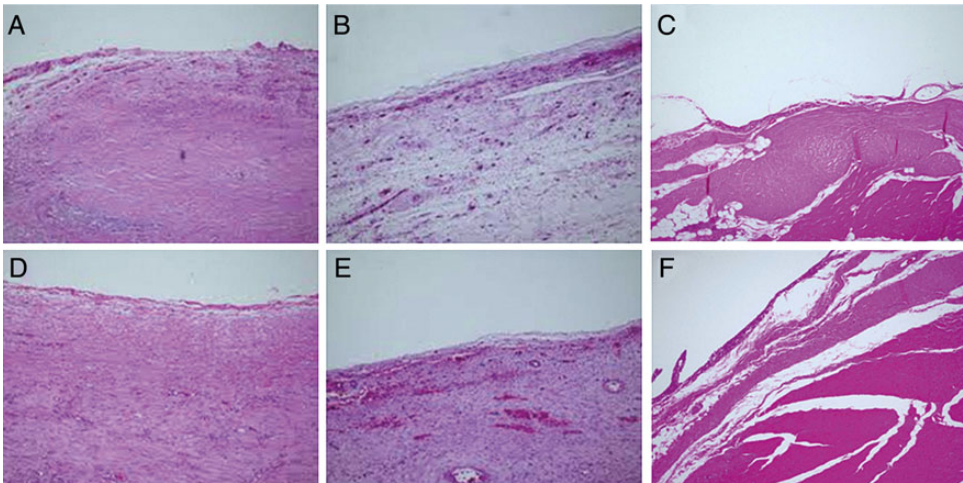


Figure 6: Representative microscopic images are shown from the same specimens as in Fig. 5. The sections of regenerated chest wall tissue were compared between the control side at 1 (A), 3 (B) and 6 (C) months and the experimental side at 1 month (D), 3 months (E) and 6 (F) months (haematoxylin and eosin stain; original magnification ×10). Regenerated mesothelial layers and no granulation tissue were observed in all specimens. Mucosal and submucosal layers appeared thicker at 1 month (a and d) and 3 months (b and e) than at 6 months (c and f) on both sides.

In previous experimental studies, powder-type bioabsorbable materials showed a relatively low rate of preventing intrapleural adhesions at 25–67% [14, 16], while the sheet-type material showed a more reliable effect (almost 100%) [12, 13]. Powder-type ones are applied on the visceral pleura with the lung deflated, and therefore, it is necessary to estimate the area of the deflated lung abutting the parietal pleural defect. As noted in our experiment, it is easy to put stitches on sheet-type ones, and thereby they stabilize on the parietal pleura. Given the ease of bringing the sheet intracorporally through a small incision, the sheet will work for video-assisted thoracoscopic surgery as well as open thoracotomy.

The aims of our study were to evaluate the safety of placing the sheet in the pleural space, to investigate the effect of the sheet on adhesion prevention in a large animal model, and to estimate the intervals during which intrapleural adhesions are formed (on the control and the experimental sides) and during which the sheet is absorbed (on the experimental side).

The safety of the sheet and its effect on adhesion prevention were demonstrated in our canine model. The effect may be associated with the relation of the two intervals (one of adhesion formation and the other of sheet absorption). The interval of adhesion formation is difficult to generalize. The discordance between a murine model and a large animal model in the effect of the same materials on preventing adhesions might be attributed to different intervals of adhesion formation between the models [14, 23]. In this experiment, all the intrapleural adhesions (on the control side) were formed within 1 month and no additional adhesion was formed nor any adhesion regressed at 3 or 6 months, which is a finding similar to previous ones [14, 23]. The findings suggested that bioabsorbable sheets are required to be sustained intrapleurally for more than 1 month to prevent adhesions in a large animal model. Hyaluronic acid-carboxymethylcellulose membrane and ϵ -poly (L-lysine) powder are not ideal materials in this setting, given that both are absorbed within 4 weeks [16]. The findings of exploratory thoracoscopy showed that the sheet was almost intact at 1 month and almost absorbed by the visceral or parietal pleura at 3 months, which is a sufficient interval for the parietal pleurae to regenerate and fill the defect microscopically as well as macroscopically.

Of interest, the bioabsorbable sheet never adhered to the visceral pleura in our experiment and was gradually absorbed by the parietal pleura without impairing healing of the parietal pleura. The reason for the different behaviours between the parietal pleura and the visceral pleura is only speculative but might derive from different phenotypes in cell lineage between visceral and parietal mesothelia [24] and/or static versus dynamic movements against the sheet.

Biochemical mechanisms in adhesion formation were not evaluated in this study and remain only speculative. In the peritoneal adhesion, type 1 plasminogen activator inhibitor, by suppressing tissue plasminogen activators, may play a role in deposition of fibrins [25]. The mechanism may apply to intrapleural adhesion formation due to the mesothelial cell lining in both the pleural cavity and the peritoneal cavity, while an inflammatory reaction itself plays a role in regenerating pleural mesothelial cells by fibroblasts, which is promoted by a basic fibroblast growth factor released from macrophages [24]. The number of capillary vessels in the mucosal and submucosal layers at the thoracotomy site was significantly greater on the experimental side than on the control side, at 1 month. The sheet might have induced an intense inflammatory reaction with neo vascularization in the same way as

collagen fleece [12], promoting pleural mesothelial regeneration while separating from the visceral pleura. On the other hand, there was no significant difference in neo vascularization between the sides at 3 and 6 months, which suggested that the absorption process did not induce chronic inflammatory reactions.

Limitations in this study included no postoperative drainage with a chest tube, although it is almost routinely performed in thoracic procedures. Also, no pulmonary resections were performed in our model. The animal model was not that of (extensive) pleuritis, in which the sheet may not work well. Biochemical analysis, which also may be helpful in following up the inflammatory reaction related to the adhesion formation, was not performed. Histological analysis was not performed for the visceral pleura because of the difficulty in identifying the area abutting the bioabsorbable sheet.

In conclusion, the bioabsorbable sheet composed of poly (L-lactide-co- ϵ -caprolactone) and polyglycolic acid felt may prevent intrapleural adhesions following thoracotomy without adverse findings. Patients who potentially require reoperative procedures in the future may benefit from bioabsorbable sheet placement at the initial thoracotomy.

Conflict of interest: none declared.

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eComment. Can this material be used for small chest wall resections?

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We have read the article by Hamaji *et al.* [1] and thank them for this meticulous study. We want to point out the possible usage of this material in limited chest wall resections.

As mentioned in the passage, intrathoracic adhesions may increase the rate of complications. Whenever the pleura is opened, there is a high risk of adhesion, regardless of the operation. Even in the tube thoracostomy, adhesions can be seen. We want to share our case involving dense adhesions [2]. We resected a rib segment and used a Prolene mesh for the chest wall. It was reported as R0 chondrosarcoma. The tumour cells were millimetres away from the margin. To enlarge the resection margins, we reoperated the patient again, about one month after the first operation. There were dense adhesions to the chest wall and to the reconstruction material. A part of the lung was injured during dissection. This complication extended the hospital stay.

In any case, adherent lung to the chest wall is prone to complications. Using materials that have low potency for adhesion will be very advantageous in all kind of pleura-related thoracic interventions, including chest wall resections. We thank the authors again and hope to see the study supported by clinical trials.

Conflict of interest: none declared.

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