In Vivo Comparative Assessments on Pleural Adhesive Effects of Three Commercially Available Sealants

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Footnotes

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Glossary of abbreviations:

PEG/HSA (polyethylene glycol and human serum albumin), PGA (bioabsorbable polyglycolic acid felt)

Central picture: Box plots showing the adhesion score, and the severity the pathologic inflammation score.

Central message: The degree of adhesion/inflammation of the three commonly used sealants was compared through an in vivo model, PEA/HSA showed least degrees of adhesion/inflammation compared to Fibrin and PGA

Perspective statement: Our results show that PEG/HSA has theoretical advantages over other materials in patients with impaired lung function with respect to high risk of recurrent chest surgery or minimal adhesion formation and the most adaptive lung dilatation.
Abstract

Objectives
The surgical sealant, used for the reinforcement of suture-lines, has been widely used in lung resection surgeries in the aim of reducing postoperative morbidity; however, it may exacerbate surgical site adhesion leaving risks of restrictive thoracic movement and difficult entrance for redo-operation. We aimed to assess the pleural adhesive effects of three frequently used surgical sealants, (1) fibrin glue (Fibrin), (2) a composite of polyethylene glycol and human serum albumin (PEG/HSA), and (3) bioabsorbable polyglycolic acid felt (PGA) in an in-vivo setting.

Methods
Eighty-one rats were randomly assigned to three experimental groups—Fibrin, PEG/HAS, and PGA groups. Following intra-pleural application of the sealants, the extent and severity of adhesion and inflammation were quantitatively compared among the 3 groups at 2, 4, and 8 weeks.

Results
The scores for both the extent and severity of adhesion were significantly higher in the PGA group than the other two groups throughout postoperative period (P<0.001 for all). Although both scores in the PES/HAS and Fibrin groups were 0 at 2 weeks, the Fibrin group showed significantly high scores than the PES/HAS group thereafter (P<0.001 for all). Trends in inflammation scores were analogue of those of adhesion scores favoring the PES/HAS group followed by the Fibrin group (P<0.001 for all).

Conclusion
Among 3 commonly used sealants, PEA/HSA showed least degrees of adhesion/inflammation compared to Fibrin and PGA, while PGA demonstrated greatest degrees of
adhesion/inflammation throughout a postoperative course of 8 weeks in an in-vivo model.

Abstract word count:237

**Key words:** Sealants; postoperative adhesion; lung resection
Introduction

In lung resection surgery, resection margins are the major source of postoperative air leaks; hence the buttressing of such suture- or staple-lines is widely used practice to mitigate postoperative air leakage. For this, a number of sealants have been developed and used in practice, and among them, 3 commercially available sealants—polyglycolic acid sheet (PGA), fibrin glue (Fibrin) and polyethylene glycol and human serum albumin (PEG/HSA)—might have been the most commonly used ones, which have demonstrated their efficacies through a number of studies[1-7]. PGA is generally a wound surface covering agent reinforcing the wounds or suture-lines effective for secondary healing [8, 9]. Fibrin is composed of human fibrinogen and human thrombin in separated chambers, which form a cross-linked gelatin-mixture by hydrogen bonding once they are exposed to each other on the applied surface [5, 10]. polyethylene glycol and human serum albumin (PEG/HSA) polymerizes to form a flexible, cross-linked hydrogel matrix, which adheres to lung tissue and allows it to stretch as the lung is reinflated [2, 11].

Among potential pitfalls of these sealants is the adhesion formation in the pleural cavity[8, 12-15]. Pleural adhesion, with intensifying extent and severity, is known to cause restrictive thoracic movement, chronic chest pain or discomfort, and consequent prolonged hospitalization and increased incidence of hospital readmissions. In addition, redo-operation, not infrequent episodes in thoracic oncologic surgeries, can be challenged in the presence of pleural adhesion by difficult entrance to the pleural cavity, poor exposure of the operative fields and a long operative time.

In these regards, research on comparative effects on pleural adhesion among these widely used commercially available sealants are important to optimize the selection for lung resection surgery. In the present study, therefore, we sought to assess the pleural adhesive effects of three
frequently used surgical sealants, Fibrin, PEG/HSA, PGA in an in-vivo setting.

**Material and Methods**

**Animals**

Non-pregnant 8-week-old female Sprague-Dawley rats weighing around 200g were used for the experiment. All rats were housed separately and maintained under standard specific pathogen-free conditions (light-dark cycle: 12:12 hours, mean temperature: 23 degrees Celsius, and mean humidity: 50%). Standard laboratory rodent chow and water were available ad libitum. On the day of the experiment, the health status of all rats was checked (diarrhea, unusual fur [loss or dirtiness], mucous discharge from the eyes or anus, and emaciation). The Pusan National University Hospital Animal Experimentation Committee approved the experiment (PNUH-2023-214) on February 7th, 2023 and all surgical procedures and anesthesia protocols were conducted in accordance with the Animal Care Guidelines of Busan National University Hospital.

**Experimental design**

Eighty-one rats were randomly assigned to 3 experimental groups—the Fibrin, PEG/HAS and PGA groups—with 27 rats in each group. Within each group, rats were further randomly allocated into 3 sub-groups (9 in each), so that they can be examined by surgical re-exploration at three different time points of 2, 4, and 8 weeks postoperatively. The adhesive effects and the inflammatory changes of the applied areas were compared among the Fibrin, PEG/HSA and PGA groups at each period. The extent and severity of adhesion were graded based on the adhesion grading scale and inflammation scores were also recorded according to the histologic scoring system (Table 1).

**Material and Surgical Technique**
All operations were performed under sterile conditions and by a single surgeon (HYA). Rats inhaled 5 mg sodium pentobarbital diluted in 1 mL of physiological saline solution. Under general anesthesia, rats, fixed in the dorsal position, received a 2-mm horizontal incision along the rib. The 6-French Nelaton catheter (width, 2 mm) designed to cut in one third to reduce internal dead space was placed at the apex of the right lung through the incision, which could be viewed by a C-arm X-ray examination (Fig. 1).

Fibrin (Tisseel™, Baxter Healthcare Corp., Deerfield, Illinois, USA) and PEG/HSA (Progel™, Bard Davol, Warwick, NY, USA) were used according to the manual provided by the manufacturer in the form of two mixtures. Fibrinogen (0.5 cc) and thrombin (0.5 cc) of the Fibrin group, and human serum albumin (0.5 cc) containing PEG crosslinker (0.5 cc) of the PEG group both in separated chambers were alternately dropped to allow the combination at the lung surface in the apex, which was followed by a small amount of air injection to remove the remaining material in the catheter. The PGA (NEOVEIL®, Gunze, Kyoto, Japan) was cut into square sheets, 5 mm × 5 mm in size, and rolled up to administer using a sharp mosquito through a 2mm incision on the chest wall. The incision was repaired with 4/0 polyvinylidene fluoride monofilament sutures.

Assessment of adhesion effect

We classified adhesion extent into five grades, based on a modified version of the classification system reported by Hiroyuki et al: grade 0, no adhesions; grade 1, ≤25 mm of the site involvement; grade 2, ≤50 mm of the site involvement; grade 3, ≤75 mm of the site involvement; grade 4, ≤100 mm of the site involvement. We classified adhesion severity into four grades, as described above: grade 0, no adhesions; grade 1, adhesions fall apart; grade 2, Adhesions can be lysed with traction; grade 3, Adhesions requiring dissection.

Pathological examination
After evaluating the adhesion extent and severity at 2, 4, 8 weeks, we excised the lobe covered with materials and immersed them in 10% formalin. We performed hematoxylin-eosin staining to evaluate severity of inflammation. We classified inflammation into four grades, based on a modified version of histologic scoring system of inflammation reported by Margaret et al: grade 0, no inflammation; grade 1, mild Inflammatory infiltration; grade 2, moderate Inflammatory infiltration; grade 3, mild Inflammatory infiltration; grade 4, moderate to severe Inflammatory infiltration. [17]

**Statistical Analysis**

The statistical analyses were performed using R Studio Version 2023.12.0. All parameters are reported as mean ± standard deviation. To compare adhesion scores (extent and severity) and microscopic inflammation in the 3 groups, Levene's test for equality of samples was used and a one-way analysis of variance (ANOVA) with a Tukey post hoc test was followed. A value of $P< 0.05$ was considered to be statistically significant.

**Results**

There were no cases of mortality or complications prior to re-exploration for examinations.

**Macroscopic Findings of the Adhesive Effects**

Regarding the severity of adhesion, PEG/HAS group showed scores of 0 in all cases throughout 2 to 8 weeks, whereas PGA group showed the highest scores of 1.67 ± 0.58, 2.33 ± 0.58, 3.00 ± 0.00 at 2, 4, and 8 weeks, respectively, which were significantly higher than those in the Fibrin and the PEG/HSA groups ($P<0.01$ for all; Table 2; Fig. 2). At 2-week, the scores for extent and severity of adhesions were not significantly different between the Fibrin and the PEG/HSA groups, however, they were significantly higher in the Fibrin group than the PEG/HSA group thereafter ($P<0.001$ for all; Table 2, Fig.2).
**Gross and pathologic inflammation scale**

Gross appearance of the affected pleural cavity in the PGA group at 8-week indicated tight fibrous capsule formation between the visceral pleura and right atrium (Fig. 4F), which could not be dissected by traction. In the Fibrin group, we observed loose filmy adhesion partially covering the affected areas at 4-week (Fig. 4A), after which multiple linear adhesion bands were observed at postoperative 8-week (Fig. 4B). No cases in the PEG/HSA group, however, showed any sign of gross adhesion formation (Fig. 4C, 4D).

Pathological study at 2-week in the PGA group showed the pathologic inflammation score of 1.33 ± 0.58, which was significantly higher than the score of 0.67 ± 0.58 in the Fibrin group (P<0.01; Table 1;Fig.3). The Fibrin group, however, showed the highest scores of 2.33 ± 0.58, 2.67 ± 0.58 at 4 and 8 weeks, respectively, which were significantly higher than those in the PGA and the PEG/HSA groups (Fig.3). Only in the PGA group, the foreign body reaction was found throughout 2 to 8 weeks, and at 8 week, tight adhesion between visceral and parietal pleurae hindered dissection of the pleural space (Fig. 5C, F, I). In the PEG/HSA group, on the other hand, the pathologic inflammation scores were as low as 0.33 ± 0.58, 0.67 ± 0.58, 0.67 ± 0.58 at 2, 4, and 8 weeks, respectively, which were significantly lower than those in the Fibrin and the PGA groups (P<0.01 for all; Table 2; Fig. 5B, E, H).

**Discussion**

The present study evaluated pleural adhesive effects of 3 commercially available sealants—Fibrin, PEG/HSA, PGA—in an in-vivo setting, and demonstrated that the use of PEG/HAS showed lower degrees of pathologic inflammation and adhesion in the pleural cavity as compared with Fibrin and PGA. There have been a number of studies on surgical materials that may inhibit adhesion formation in the setting of sealant applications. Matoba et al.
demonstrated that anti-adhesive effects of non-cross-linked alginites were effective for preventing PGA-induced adhesions [18]. Takagi et al. presented comparable anti-adhesive effect of the material composed of aldehyde dextran and ε-poly powder to prevent the postoperative pleural adhesion [19]. The basic premise of these studies entails the substantial adhesive effects in most commercially available sealants for thoracic surgery, and therefore, assessments of the sealants on pleural adhesive effects per se are also clinically important.

The strengths of this study include providing a comprehensive view of the extent and severity of pleural adhesion, and pathologic lung inflammation induced by the sealants. In particular, the severity of adhesion in the PGA group showed the highest scores throughout 2- to 8-week time points, which were significantly higher than those in the Fibrin and the PEG/HSA groups, and parietal pleura adhered tightly to the visceral pleura at 8-week point that could not be dissected by traction. As the hydrolysis of PGA to glycolide results in the inflammatory responses in implanted tissues [20], a substantial level of inflammation found in histologic examinations of lung tissue in the present study correlates well with its underlying action mechanism PGA (Fig 5I).

Meanwhile, the pathologic inflammation scores in the Fibrin group showed the highest scores at 8 weeks, which was even higher than that in the PGA group (Table 2, Fig.3). This seems not matching well with lower levels of adhesion formation of the Fibrin group as compared with the PGA group at this timepoint. One prior study, however, demonstrated similar results with those of the present study that fibrinogen/thrombin compound only induce a pronounced local inflammatory response to lung parenchyme promoting tissue healing but without leaving significant residual adhesion [21].

On the other hand, the severity of adhesion in the PEG/HSA group showed scores of 0 in all cases throughout 2- to 8-week timepoints, and no cases showed a sign of gross adhesion
formation. Moreover, the pathologic inflammation scores throughout 2- to 8-week timepoints were significantly lower than those in the Fibrin and the PGA groups.

This may be explained by the reversible dynamic mechanics of PEG/HSA, in that PEG hydrogel gives rise to adhesions only in contact with moist, deformable tissue while the hydrogel becomes flexible at elevated pH of human body ranges [22, 23]. Attributed to these bimodal mechanisms of PEG/HSA, it maintains a highly compliant and flexible feature while offering sealant/adhesive capacities on the lung surface. Furthermore, PEG hydrogel coating is known to shield the biomaterial surface and thereby passively restricts complement activation to prevent inflammation [22]. These working mechanisms of PEG/HSA might have contributed to minimum levels of inflammation and adhesion in the present study. Based on the study findings, PEG/HSA seems to have theoretical benefits over other materials in patients with high risks of repeated thoracic surgeries, or with impaired lung function in the respect of least adhesion formation and most compliant lung expansion.

Limitations

The results demonstrated by the present experiment are derived from a rat model, and therefore, the reproducibility of the findings should be further validated by human clinical trials. Due to the lack of assessing the efficacy of air leak in this model, further studies should be followed to evaluate the differential air-sealing effects of the sealants.

Conclusions

Among 3 commonly used sealants, PEA/HSA showed least degrees of adhesion/inflammation compared to Fibrin and PGA, while PGA demonstrated greatest degrees of adhesion/inflammation throughout a postoperative course of 8 weeks in an in-vivo model.
**Reference List**


Table 1. Category and description of adhesion and inflammation

<table>
<thead>
<tr>
<th>Category and description</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Macroscopic</strong></td>
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<tr>
<td><strong>Extent</strong></td>
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<tr>
<td>No involvement</td>
<td>0</td>
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<tr>
<td>≤25 mm of the site involved</td>
<td>1</td>
</tr>
<tr>
<td>≤50 mm of the site involved</td>
<td>2</td>
</tr>
<tr>
<td>≤75 mm of the site involved</td>
<td>3</td>
</tr>
<tr>
<td>≤100 mm of the site involved</td>
<td>4</td>
</tr>
<tr>
<td><strong>Severity</strong></td>
<td></td>
</tr>
<tr>
<td>No adhesion present</td>
<td>0</td>
</tr>
<tr>
<td>Adhesions fall apart</td>
<td>1</td>
</tr>
<tr>
<td>Adhesions can be lysed with traction</td>
<td>2</td>
</tr>
<tr>
<td>Adhesions requiring dissection</td>
<td>3</td>
</tr>
<tr>
<td><strong>Microscopic</strong></td>
<td></td>
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<tr>
<td><strong>Histologic scoring system of inflammation</strong></td>
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<tr>
<td>Normal</td>
<td>0</td>
</tr>
<tr>
<td>Mild Inflammatory infiltration</td>
<td>1</td>
</tr>
<tr>
<td>Moderate inflammatory cells infiltration</td>
<td>2</td>
</tr>
<tr>
<td>Moderate to severe inflammatory cells infiltration</td>
<td>3</td>
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Table 2. The adhesion extent, severity scores and inflammation scores

<table>
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<tr>
<th></th>
<th>PEG/HSA</th>
<th>Fibrin</th>
<th>PGA</th>
<th>p-value</th>
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<td><strong>Adhesion Extent</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>2 weeks</td>
<td>0.00</td>
<td>0.00</td>
<td>1.67 ± 0.57</td>
<td>&lt; 0.01</td>
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<tr>
<td>4 weeks</td>
<td>0.00</td>
<td>0.67 ± 0.57</td>
<td>2.67 ± 0.57</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>8 weeks</td>
<td>0.00</td>
<td>1.00 ± 0.00</td>
<td>2.67 ± 0.58</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td><strong>Adhesion Severity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 weeks</td>
<td>0.00</td>
<td>0.00</td>
<td>1.67 ± 0.58</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>4 weeks</td>
<td>0.00</td>
<td>0.67 ± 0.58</td>
<td>2.33 ± 0.58</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>8 weeks</td>
<td>0.00</td>
<td>0.67 ± 0.58</td>
<td>3.00 ± 0.00</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td><strong>Inflammation</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>2 weeks</td>
<td>0.33 ± 0.58</td>
<td>0.67 ± 1.33 ± 0.58</td>
<td>&lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>4 weeks</td>
<td>0.67 ± 0.58</td>
<td>2.33 ± 1.67 ± 0.58</td>
<td>&lt; 0.01</td>
<td></td>
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<tr>
<td>8 weeks</td>
<td>0.67 ± 0.58</td>
<td>2.67 ± 1.67 ± 0.58</td>
<td>&lt; 0.01</td>
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**Figure legends**

Figure 1. X-ray of rat. The tip of catheter (arrow) could be seen at the apex of the right lung.

Figure 2. Box plots showing the adhesion score after 2 weeks (A), 4 weeks (B), 8 weeks (C) and the severity after 2 weeks (D), 4 weeks (E), 8 weeks and (F) after application of a Fibrin glue, a Polyethylene Glycol and Human Serum Albumin (PEG/HSA), and a Polyglycolic acid (PGA), respectively.

Figure 3. Box plots showing the pathologic inflammation score after 2 weeks (A), 4 weeks (B), 8 weeks (and C) after application of a Fibrin glue, a Polyethylene Glycol and Human Serum Albumin (PEG/HSA), and a Polyglycolic acid (PGA), respectively.

Figure 4. Gross intraoperative findings. A, B, C, D, E, F represent a polyethylene glycol and human serum albumin (PEG/HSA) group (A, B), a fibrin glue (Fibrin) group (C, D), a polyglycolic acid (PGA) group (E, F), and 4 weeks (A, C, E), 8 weeks (B, D, F) after application, respectively.

C. Diffuse adhesion (arrow) could be seen. D. Multiple linear adhesion band (arrow) exist, A, B. No adhesion, E. A strong adhesion (arrow) has been developed between the parietal pleura and lung, F. The strong adhesion aggravated the foreign body reaction (arrow) over 8 weeks, which could be seen between the visceral pleura and right atrium.

Figure 5. Hematoxylin and eosin staining of lung tissue. Original magnification ×40. A, B, C, D, E, F, G, H, and I represent a polyethylene glycol and human serum albumin (PEG/HSA) group (A, D, G), a fibrin glue group (B, E, H), and a polyglycolic acid (PGA) group (C, F, I), 2
weeks (A, B, C), 4 weeks (D, E, F), and 8 weeks (G, H, I) after application, respectively.

A PEG/HAS group: A, D, G. Few inflammatory cells infiltrate without any differences among
time differences. A fibrin glue group: B. Mononuclear inflammation cells infiltrate. E. Diffuse
inflammatory cells and multinucleated giant cells (asterisk) are infiltrated. H. Large number of
diffuse inflammatory cells and multinucleated giant cells(asterisk) infiltrate. A PGA group: C.
A foreign body reaction (red double head arrow) forms in response to the introduction of
exogenous material, and amorphous materials are filled (black double head arrow) between
foreign body reaction and visceral pleura with slight infiltrated lymphocytes (arrow). F.
Mononuclear inflammation cells infiltrate around thickened visceral pleura (triangle). I.A
foreign body reaction (red double head arrow) let parietal pleura (arrow) be stuck together and
visceral pleura (triangle) be more thickened.