Bronchoscopic treatment of postpneumonectomy bronchopleural fistula with a collagen screw plug

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Objective: Bronchopleural fistula is a critical complication that may occur after pulmonary resection. Early closure of the fistula is required to prevent thoracic empyema or aspiration pneumonia. We have designed a novel procedure for bronchoscopic occlusion of the fistula with a collagen screw plug and assessed its feasibility in an experimental animal model.

Methods: Adult beagle dogs underwent right or left pneumonectomy, and the bronchial stump was closed with the Sweet method. A silicone bar (2 mm in diameter) was then placed in the middle of the bronchial stump. Seven days after the operation, the silicone bar was removed bronchoscopically, and fistula formation was confirmed. A screw-shaped 2% collagen screw plug (20 mm long and 3 mm in diameter) was mounted at the end of a modified endoscopic cannula and then inserted into the fistula. Autologous platelet-rich plasma was then soaked onto the inserted plug.

Results: Nine of 10 beagle dogs with bronchopleural fistula were treated successfully by plug occlusion. One dog died of pneumothorax caused by dislocation of the plug. Pathologic examination revealed that the collagen sponge had been replaced by fibrous tissue and that the fistula was covered with normal epithelium. Although soaking with platelet-rich plasma made the plug airtight immediately, the use of platelet-rich plasma seemed to make no distinct difference with respect to the treatment result or pathologic findings.

Conclusion: Bronchoscopic occlusion with a collagen screw plug is a promising option for treatment of small bronchopleural fistulas after pulmonary surgery.

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Bronchopleural fistula (BPF) is a life-threatening postoperative complication seen in 0.8% to 15% of patients undergoing pneumonectomy.^{1,2} Chest tube drainage and early closure of the fistula are required to prevent subsequent aspiration pneumonia, which is associated with high mortality.³ With recent advances in bronchofibroscopy, bronchoscopic treatment of small BPFs with thoracic drainage has come to be considered as first-line therapy.^{4,5}

Although fibrin sealants are widely used to occlude BPFs, the use of such sealants requires multiple interventions and often yields unsatisfactory results.⁶⁻⁸ Expectoration of fibrin sealants causes failure of BPF closure and is thus a major concern for clinicians.⁴ For a few minutes immediately after mixing of the two

Abbreviations and Acronyms

BPF = bronchopleural fistula

PRP = platelet-rich plasma

components, fibrin sealants lack sufficient strength. Furthermore, it is clinically difficult to maintain complete apnea for several minutes to allow the fibrin glue to solidify into a stable fibrin clot. We therefore expected that occlusion with a screw-shaped plug a little larger than the diameter of the fistula would solve this problem.

Collagen, a basic component of connective tissue, is biodegradable and biocompatible. Because collagen promotes cellular proliferation and tissue healing,⁹ it has been widely used as a scaffold for tissue regeneration in the field of in situ tissue engineering.¹⁰⁻¹²

In this study, we compared the effectiveness of a screw-shaped collagen sponge plug with that of fibrin sealants *ex vivo* as an occlusive material. We then examined the feasibility of using a collagen screw plug in an animal BPF model, in which the additive effect of platelet-rich plasma (PRP) for promotion of the healing process was also evaluated.

Material and Methods**Preparation of Collagen Screw Plug**

A screw-shaped collagen sponge plug (20 mm long and 3 mm in diameter) was manufactured. Briefly, a 2% collagen hydrochloride solution (Nippon Meat Packers, Inc, Osaka, Japan) was pigmented yellow with food coloring to make it visually distinguishable from surrounding tissue during the treatment procedure. The solution was poured into a screw-shaped mold and then freeze-dried for 12 hours (Ulvac GCD135XA; Sinkukiko Ltd, Tokyo, Japan), followed by 24 hours of dehydrothermal treatment at 140°C in a vacuum to induce cross-linkage between the collagen molecules. The resulting plug, which had shrunk as a result of the freeze-drying treatment, was then replaced in the screw-shaped mold. To improve the mechanical properties of the plug, 2% collagen solution was poured over it, and the freeze-drying and dehydrothermal treatments were repeated. Finally, the plug was sterilized with ethylene oxide gas and stored dry until use. The completed screw plug is shown in Figure 1.

Assessment of Screw Plug Characteristics

Before the experiment with the animals, the characteristics of the plug as an occlusive material were assessed *ex vivo*. As a fistula model, a silicone cylinder 3 cm in height with one end closed and the other end open was prepared. A hole 2 mm in diameter, to act as a BPF, was created at the center of the closed end. The hole in the cylinder was occluded with either a screw-shaped plug or commercial fibrin glue (Bolheal; Kaketsuken, Kumamoto, Japan) applied by the double-layer method. Two types of screw plug were used, one kept dry and the other wetted with 0.2 mL saline solution ($n = 10$ each). The open end of the cylinder was connected to a

Figure 1. A, Collagen screw plug (lower) and metal screw used to create mold (upper). B, Scanning electron micrograph of screw plug shows pore structure of collagen sponge.

ventilator, and the air pressure was gradually increased from 0 to 60 cm H₂O until air leakage or material dislocation was observed. In the case of fibrin glue, air pressure was applied 1, 1.5, 2, and 3 minutes after mixing of the two components ($n = 8$ each). Results are expressed as mean $\pm$ SD.

Creation of BPF Model

Thirteen adult beagle dogs weighing 7.8 to 13.2 kg were anesthetized by intramuscular administration of ketamine hydrochloride and xylazine and then intubated with a 35F endobronchial tube (Broncho-Cath; NCC Division Mallinckrodt, Inc, Argyle, NY). Respiration was maintained by a mechanical ventilator with halothane and nitrous oxide gas. A right or a left pneumonectomy was performed through a thoracotomy in the fifth intercostal space. The main bronchus was sharply transected close to the second carina, and the bronchial stump was closed with the Sweet method¹³ with 3-0 Prolene suture (Ethicon, Inc, Somerville, NJ). To create a

fistula at the bronchial stump, a silicone rod 2 mm in diameter and 20 mm long, later to be removed, was placed in the middle of the bronchial stump. Ampicillin sodium–cloxacillin sodium (500 mg/body) was administered intramuscularly just before and on the day after pulmonary resection.

Seven days after the pneumonectomy, the dogs were intubated with a 9F tracheal tube under general anesthesia. An 8F chest tube was inserted into the postpneumonectomy space and was left on water seal. The following procedures were performed under spontaneous breathing. The silicone rod that had been placed in the center of the bronchial stump was removed bronchoscopically (Figure 2, A), and the presence of a fistula was confirmed by inserting a metal probe into the thoracic cavity under radiographic guidance. Fistula formation was also confirmed by air leakage from the chest tube.

Bronchoscopic Treatment

Immediately after removal of the silicone rod, a collagen screw plug was mounted at the end of an endoscopic cannula that had been modified by removing the brush head from a pair of brushing bioptic forceps (BC-9C; Olympus Optical Co, Ltd, Tokyo, Japan). The plug was then inserted into the fistula through the bronchofibroscop (Figure 2, B). For each animal, 5 mL of peripheral blood was centrifuged at 2400 rpm for 10 minutes to obtain PRP. In 6 of 10 dogs, 1 mL of autologous PRP was soaked onto the inserted screw plug through the sheath of the endoscopic cannula just after occlusion of the BPF. Disappearance of air leakage through the chest tube was confirmed, and then the chest tube was removed.

Bronchofibrosopic observation was carried out on days 3, 7, 10, 14, and 28 after the occlusion procedure. Additional resection of the stump was performed 4 weeks after the treatment for histologic assessment. This animal experiment was performed in accordance with the Guidelines for Animal Experiments of Kyoto University (1989).

Histologic Analysis

Specimens of the bronchial stump were fixed in 10% formalin and embedded in paraffin. Sections 4 μ m thick were stained with hematoxylin-eosin and the standard Masson trichrome method and then examined microscopically.

Results

Characteristics of Screw Plug

In the silicone cylinder model, the dry screw plug retained its air-tightness to an air pressure of 16.8 ± 7.0 cm H₂O, whereas the wetted screw plug did so to 34.5 ± 11.7 cm H₂O. Both the dry and wetted screw plugs retained their position to an air pressure of 60 cm H₂O. Commercial fibrin glue prepared by mixing two components and left for less than 1.5 minutes became dislocated and was unable to retain airtightness at an air pressure of 5 to 10 cm H₂O, whereas glues left for more than 2 minutes showed neither dislocation nor air leakage to an air pressure of 60 cm H₂O.

Treatment Outcome

Thirteen beagle dogs were subjected to surgery, and total of 13 BPFs (6 on the right, 7 on the left) were created (Table 1).

Figure 2. A, BPF just after removal of silicone bar. B, Screw plug (small arrow) inserted into BPF from end of endoscopic cannula (large arrow). C, Findings on 14th day after treatment. BPF is completely covered with bronchial mucosa.

TABLE 1. Results of the procedure

Dog	Sex	Body weight (kg)	Side	Treatment	Result
1	Female	10.5	Left	CSP	Cured
2	Female	12.6	Left	CSP	Cured
3	Female	11.3	Left	CSP and PRP	Cured
4	Female	7.9	Left	—	Died, postoperative day 6
5	Male	10.2	Left	CSP and PRP	Cured
6	Male	13.2	Left	—	Died, postoperative day 7
7	Female	10.0	Left	CSP and PRP	Cured
8	Female	7.6	Right	CSP and PRP	Cured
9	Male	9.0	Right	CSP and PRP	Cured
10	Female	11.0	Right	CSP	Died, treatment day 5
11	Female	9.0	Right	CSP and PRP	Cured
12	Male	9.1	Right	—	Died, postoperative day 4
13	Female	9.7	Right	CSP	Cured

CSP, Collagen screw plug.

Collagen plug occlusion was performed on 10 BPFs (5 on each side), among which PRP injection was applied to 6 (3 on each side). Three dogs (1 with a right BPF and 2 with left BPFs) died of pneumothorax within 1 week as a result of dislocation of the silicone rod before the treatment procedure. Nine of the remaining 10 dogs were cured by screw plug occlusion. One dog with a right BPF that had been treated without PRP injection (dog 10) died of tension pneumothorax on the fifth day after treatment, and autopsy revealed disappearance of the inserted screw plug.

Bronchofibroscopic Findings

At the time of silicone rod removal, mucosal edema was observed around the rod in all 10 dogs that survived to that point. The screw plug was recognizable on the third day after treatment in all dogs except dog 10 but became gradually indistinguishable from the surrounding tissue by the 10th day after treatment. By 2 weeks after the procedure, bronchial mucosa with capillary vessels covered the plug (Figure 2, C). No marked differences were observed between dogs with and without the PRP treatment. No distinct signs of airway infection or inflammation were observed throughout the experiment. In dog 10, accumulation of secretion over the inserted screw plug was observed on the third day after treatment, and we performed saline irrigation around the stump so that the inserted screw plug could be clearly observed. This procedure may have accelerated collagenolysis of the screw plug, which resulted in its dislocation from the fistula site.

Figure 3. Microscopic appearance of bronchial stump 4 weeks after treatment procedure. Arrow indicates occluded BPF. Fistula is filled with fibrous tissue and covered with bronchial epithelium. M, Bronchial smooth muscle; C, bronchial cartilage. Masson trichrome stain; original magnification $\times 100$.

Macroscopic and Histologic Findings

At rethoracotomy, no signs of infection were observed in the pneumonectomy space. The bronchial stumps were covered with fibrous tissue, and the intraluminal surface of the fistula was covered with bronchial mucosa. At 4 weeks after the procedure, the collagen screw plug was replaced by fibrous tissue and the area was covered with normal epithelium (Figure 3). Pathologically, there were no clear differences in outcome between the dogs that were treated with PRP and those that were not.

Discussion

Postpneumonectomy BPF is a life-threatening complication, and BPF closure has long been a tedious task for clinicians. Various methods have been developed, and several materials, such as fibrin glue, have been tried with the aim of closing the fistula. Although BPF closure has been performed clinically, the results are often unsatisfactory in terms of the materials or methods used. In the case of fibrin glue, which continues to be used for the first-line treatment of BPF because of its ready availability, we think that the difficulty lies in using liquid components in an attempt to occlude a space in which respiratory airflow moves.

We hypothesized that BPF occlusion with collagen, which promotes cellular proliferation and wound healing, would lead to a more favorable outcome than that seen with conventional methods. We also hypothesized that molding the occlusive material into a screw shape would help reduce the incidence of dislocation. As we expected, the collagen

screw plug used in this study effectively occluded the BPF without expectoration or dislocation into the thoracic cavity in all but 1 case. Macroscopically, the inserted collagen had disappeared 4 weeks after the treatment procedure, and the fistula became filled with connective tissue. Fibrous proliferation and epithelial covering of the BPF were also observed on pathologic examination. We therefore think that the collagen sponge acted as a scaffold for cellular proliferation and tissue healing.

Our ex vivo study showed that a wetted screw plug was more suitable for BPF closure than a dry one, maintaining airtightness at a higher air pressure. When tested in vivo, however, the wetted screw plug became too soft for effective insertion into the BPF. It thus appears essential to store the plugs in a dry state until the time of insertion. We found that an inserted screw plug gradually became moist within several minutes and provided an airtight seal for the fistula. We therefore consider the absorbency of collagen another advantage relative to conventional fibrin sealants.

With regard to growth factors, PRP, which can be easily obtained in a clinical setting, contains various growth factors important for wound healing, such as platelet-derived growth factor, transforming growth factor β , vascular endothelial growth factor, epidermal growth factor, and insulin-like growth factor.¹⁴ Because autologous PRP is a favorable substance for combined use with the screw plug, avoiding the transmission of infectious agents, we applied it for this experiment. The dry screw plug became airtight soon after application of PRP, as suggested by the immediate disappearance of air leakage through the chest tube. There were no distinct differences in either fibrous proliferation or shortening of the healing process related to PRP use. Nevertheless, PRP seemed to have no unfavorable effects on fistula closure.

In this study, we created a fistula 2 mm in diameter, because this size is commonly observed clinically.⁶ In cases of BPFs exceeding 2 mm in diameter, larger plugs may be effective. Because we thought that a plug slightly larger than the diameter of the fistula might become dislocated less readily, we used a plug 3 mm in diameter to occlude the fistula. For large BPFs, such as those 8 mm or more in diameter, reoperation would be the first choice of treatment.⁶

To our knowledge, the canine model we have created is the first of its kind reported for assessing the long-term outcome of treatment for early-onset BPF after pneumonectomy. At the beginning of this study, 3 dogs died of BPF as a result of dislocation of the silicone rod. This suggests that the method used for fixing the silicone rod to create a BPF is a critical point that may have room for improvement. Nevertheless, our canine model is reliable in that it reflects actual clinical conditions.

Clinical diagnosis of BPF is not necessarily made immediately after its occurrence and is often associated with

empyema.^{1,3} The collagen plug in this study was efficient in occluding BPF in an experimental dog model. BPFs are often found with infection and inflammation of the surrounding tissues, however, so whether the same result can be obtained in clinical BPF remains unknown. Although it would be difficult to create and maintain an empyema model with fistula formation, further investigation under conditions of infection and inflammation should be conducted before clinical application of the collagen screw plug.

The incidence of BPF after pneumonectomy is reportedly higher on the right side than on the left, probably because of the larger size of the right main bronchus and the lack of mediastinal coverage.¹⁵ This may explain why the fistula could not be closed in 1 dog with a right BPF, whereas all the dogs with a left BPF were effectively treated with the plug.

Although the reported incidence of postoperative BPF has been reduced since development of mechanical stapler,¹⁶ BPF still remains as a serious problem. We believe that our procedure is beneficial because it can be applied almost immediately after bronchoscopic discovery of BPF and requires minimal preparation and no specific skills.

Conclusions

We have created a canine model of postpneumonectomy BPF and designed a novel procedure for bronchoscopic occlusion of the fistula with a collagen screw plug. BPFs were effectively closed with the inserted screw plug, which eventually was replaced by fibrous tissue. Careful assessment is essential before clinical application, considering the difference between experimental animal models and human patients in wound healing; nevertheless, we think that bronchoscopic occlusion with a collagen screw plug is an effective therapy for a small BPF after pulmonary surgery.

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