Intra-thoracic fibrous tissue induction by polylactic acid and epsilon-caprolactone copolymer cubes, with or without slow release of basic fibroblast growth factor

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Abstract

Objective: We investigated whether implantation of polylactic acid and epsilon-caprolactone copolymer (PLAC) cubes with or without basic fibroblast growth factor (b-FGF) released slowly from gelatin microspheres was able to induce fibrous tissue in the dead space remaining after pneumonectomy in the thoracic cavity.

Methods: Left pneumonectomy was performed in Japanese white rabbits. In the control group (n = 6), the left thoracic cavity was closed without any treatment. In the FGF group (n = 6), gelatin microspheres that released 100 μg of b-FGF were implanted into the left thoracic cavity. In the PLAC group (n = 6), PLAC cubes were implanted into the left thoracic cavity. In the PLAC/FGF group (n = 6), both PLAC cubes and gelatin microspheres releasing 100 μg of b-FGF were implanted into the left thoracic cavity.

Results: In the control and FGF groups, herniation of the heart, mediastinal shift, and overinflation of the right lung were observed. No granular tissue formation was observed. In the PLAC and PLAC/FGF groups, a dense area of newly formed soft tissue was observed, and only a mild mediastinal shift was observed during the 3-month follow-up period. Pathological examination revealed induction of fibrous and granular tissue in the left thoracic cavity. The foreign-body reaction induced by PLAC was very mild.

Conclusions: Implantation of PLAC cubes with or without gelatin microspheres releasing 100 μg of b-FGF is able to induce fibrous tissue in the post-pneumonectomy dead space.

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1. Introduction

The intrapleural space remaining after pulmonary resection can cause various complications, especially in patients with pulmonary fibrosis and severe emphysema. A small number of patients may also develop empyema, which can be a critical complication in this situation. In order to reduce this dead space, thoracoplasty [1], muscle transposition [2—4], and omentopexy [5,6] are sometimes indicated. However, these procedures are associated with severe damage, thoracic deformity or discomfort attributable to laparotomy. If obliteration of the dead space can be achieved using the patient’s own fibrous tissue, then the morbidity associated with pulmonary resection may be minimized, and quality of life may be maintained. In previous studies, we have succeeded in regenerating esophageal tissue [7], tracheal tissue [8], and tracheal cartilage [9,10] using biodegradable materials and application of growth factor. Polylactic acid and epsilon-caprolactone copolymer (PLAC) is a synthetic and biodegradable material whose medical qualities and reliability are already well established. It has been reported that PLAC per se can induce angiogenesis and migration of fibroblasts and macrophages as a result of a sterile foreign body reaction [11]. Basic fibroblast growth factor (b-FGF) has many activities and has been widely employed, especially for promotion of angiogenesis and wound healing [12,13]. In the present study, we examined whether implantation of polylactic acid and epsilon-caprolactone copolymer (PLAC) cubes with or without gelatin microspheres (GMS) releasing 100 μg of b-FGF induced the formation of fibrous tissue in the dead space in the thoracic cavity remaining after pneumonectomy.

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2. Materials and methods

2.1. Preparation of PLAC cubes

Poly l-lactic acid (Gunze Co., Ltd., Tokyo, Japan) and caprolactone (Gunze Co., Ltd., Tokyo, Japan) were dissolved in dioxane (Sigma-Aldrich Laborchemikalien, GmbH, Seelze, Germany) to make a 7.5% polymer solution. Sodium chloride crystals were added to make a 97% solution, which was then cast in a mold and freeze-dried. The material was washed with double-distilled water (DDW) to remove the sodium chloride, and then dried again. The PLAC was then cut into 0.8 cm³ cubes (Fig. 1A). den Dunnen et al. have reported that ingrowths of various cells (fibroblasts, macrophages and giant cells) in PLAC bars was observed 6 months after implantation of the biomaterial [11]. To facilitate early cell migration, we gave the PLAC cubes a honeycomb structure with a pore diameter of 200—400 µm (Fig. 1B).

2.2. Preparation of b-FGF-impregnated gelatin microspheres

A 5% gelatin solution (Nitta Gelatin Co., Ltd., Osaka, Japan) was added drop wise to olive oil. The gelatin microspheres, 75—125 µm in diameter, were immersed in 0.13% glutaraldehyde (Fig. 2A). A solution of 100 µg/100 µl b-FGF (Kaken Pharmaceutical Co., Tokyo, Japan) was added to 10 mg of the dried gelatin microspheres. The FGF-GMS was suspended in 5.0 ml of physiological saline (Fig. 2B) [14]. Previous studies have shown that an implantation dose of 100 µg of b-FGF-containing gelatin microspheres is enough to induce tissue ingrowths in mouse and rat models [15,16] and that slow release of FGF was achieved for up to 15 days.

2.3. Surgical procedures

Twenty-four adult Japanese white rabbits weighing 2.7—3.7 kg (mean 3.3 kg) were used. They were anesthetized by intramuscular injection of 10 mg/kg xylazine (Selactal®, Bayer, Tokyo, Japan) and 50 mg/kg ketamine (Ketalar®, Sankyo Co., Ltd., Tokyo, Japan) with 0.1 mg/kg atropine sulfate (Fuso Pharmaceutical Industries, Ltd., Osaka, Japan). In the control group (n = 6), left pneumonectomy was performed under spontaneous respiration without any implantation, and the chest was then closed. In the FGF group (n = 6), 100 µg of b-FGF-containing gelatin microspheres were implanted into the post-pneumonectomy space, and the chest was closed. In the PLAC group (n = 6), five PLAC cubes (0.8 cm × 0.8 cm × 0.8 cm) were
implanted into the post-pneumonectomy space and the chest was closed. In the PLAC/FGF group \((n = 6)\), five PLAC cubes \((0.8 \text{ cm} \times 0.8 \text{ cm} \times 0.8 \text{ cm})\) and 100 \(\mu\)g of b-FGF-containing gelatin microspheres were implanted, and the chest was closed.

### 2.4. Follow-up studies

Routine chest computed tomography (CT) was carried out at 1, 2, and 3 months after surgery. Two rabbits in each group were killed for pathological examination at 1, 2, and 3 months after surgery in each group. The left parietal pleura and all tissues were immediately removed, fixed in 10% formalin, and then examined pathologically after hematoxylin eosin or elastica van Gieson staining. All of the animal experiments were carried out in accordance with the ‘Guide for the Care and Use of Laboratory Animals’ prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH publication No. 86; 23, revised 1985).

### 3. Results

#### 3.1. Clinical findings

All of the rabbits survived until sacrifice without any major health problems.

#### 3.2. Radiographic findings

In the control and FGF groups, mediastinal shift was observed at 1 month after surgery, and this was still evident at 3 months after surgery (Fig. 3A). No newly formed soft tissue area was observed in the thoracic cavity. In the PLAC group and the PLAC/FGF group, mild mediastinal shift was observed at 1 and 3 months after surgery. A newly formed area of soft tissue was observed at 1 and 3 months after surgery (Fig. 3B). There were no dead spaces in either of these two groups.

#### 3.3. Macroscopic findings

In the control and FGF groups, the parietal pleura was slightly thickened. However, there were no significant differences at each time point. In the PLAC and PLAC/FGF groups, thick fibrous tissue was observed around the PLAC cubes at 1 month after surgery. This appearance remained essentially unchanged throughout the observation period. The form of the cubes collapsed, as they were slowly absorbed.

#### 3.4. Microscopic findings

In the control and FGF groups, no granular tissue formation was observed throughout the observation period. Until 3 months, the inner and outer connective tissue layers were thickened because of an increase in collagen bundles (Fig. 4). These findings were similar in both groups.

In the PLAC group at 1 month, the PLAC cubes, which were covered by a fibroblast and fibrous collagen layer, maintained their volume and showed rounding off of their corners (Fig. 5A). The PLAC cubes were invaded by granular tissue through the micro pores (Fig. 5B). At 2 and 3 months, the PLAC cubes were covered by a fibrous collagen layer surrounded by adipose tissue (Fig. 5C). At 1 month, the septa of the honeycomb structure of the PLAC cubes were observed among the invaded inflammatory cells and fibroblasts in the peripheral areas of the cubes. The pores of the honeycomb structure were occupied by collagen fibrils and fibroblasts (Fig. 5D). At 2 and 3 months, the honeycomb structure was no longer evident. Collagen fiber deposition was observed in all areas that appeared to correspond to the original pores. The foreign-body reaction due to PLAC was minimal and limited.

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Fig. 3. Chest computed tomography (CT) findings. No soft tissue area was detected in the post-pneumonectomy space in the control group at 1 month (A) and 3 months (C) after surgery. High-density area was detected in the post-pneumonectomy space in the PLAC group at 1 month (B) and 3 months (D) after surgery. PLAC: polylactic acid and epsilon-caprolactone copolymer.

Fig. 4. Microscopic findings in the control group, showing slight thickening of the parietal pleura. No substantial fibrous tissue was detected. In the control group and FGF group, no granular tissue formation was evident throughout the observation period.
In the PLAC/FGF group at 1 month, the PLAC cubes were covered by fibroblasts and a fibrous collagen layer, and maintained their shape. At 2 and 3 months, the PLAC cubes were covered by adipose tissue and filled with granular tissue, showed a reduction in volume and were rounded off at their corners. All of the findings in this group were similar to those in the PLAC group.

4. Discussion

A good rule for the thoracic surgeon is ‘no space, no problem’ [17]. The dead space remaining after lung resection is usually obliterated by the over-inflated remaining lung. However, if the patient has pulmonary fibrosis or severe emphysema, a large dead space sometimes persists after surgery. In addition, if these patients develop empyema and bronchopleural fistula, their condition can occasionally become refractory or critical. In order to reduce this dead space, thoracoplasty [1], muscle transposition [2—4] and omentopexy [5,6] have been attempted, and reportedly became refractory or critical. In order to reduce this dead space, thoracoplasty [1], muscle transposition [2—4] and omentopexy [5,6] have been attempted, and reportedly effective. However, these procedures carry a moderate risk and have the potential to cause severe damage. Therefore, obliteration of the dead space using the patient’s own fibrous tissue would help to maintain quality of life.

In previous studies we have succeeded in regenerating esophageal tissue [7], tracheal tissue [8], and tracheal cartilage [9,10] using biodegradable materials and growth factors. Therefore, encouraged by our results, we tried to induce the formation of fibrous tissue in the thoracic cavity by tissue engineering. We intended to perform intra thoracic thoracoplasty with self-organization of tissues using a biodegradable material and growth factor. In our previous study, we proved that a gelatin sponge was able to act as a carrier of cytokine [10]. It has been reported that PLAC as a scaffold can itself induce angiogenesis, migration of fibroblasts and generation of a collagen layer [11]. We considered that a combination of slow cytokine release from a carrier, together with a scaffold, was essential. However, in this study, migration of fibroblasts and generation of a collagen layer was achieved using PLAC cubes either with or without FGF. In the control and FGF groups, mediastinal shift was observed but no fibrous tissue developed during the 3-month follow-up period. On the other hand, in the PLAC and PLAC/FGF groups, a mild mediastinal shift was observed, and newly formed soft tissue developed during the 3-month follow-up.

Pathologically, thick fibroblast layers were observed around the PLAC cubes at 1 month after surgery in the PLAC and PLAC/FGF groups. By 3 months, the collagen layers had spread widely and deeply, and ingrowths of cells (fibroblasts, macrophages and giant cells) into the cubes was achieved. This sequence of events suggests that the fibrous tissue was induced by PLAC with micro pores with or without gelatin microspheres that released 100 μg of b-FGF. It has been reported that ingrowths of cells into rigid PLAC bars was observed 6 months after their implantation [11]. For the early facilitation of cell migration, we created 200—400 μm micro pores in the PLAC cubes, and this achieved both early cell migration and collagen generation. The cubes fabricated from the PLAC were biodegradable, permeable, and biocompatible. This material is widely used as a scaffold for nerve regeneration [18—21].

The addition of 100 μg/10 mg FGF-GMS did not accelerate intra thoracic tissue generation. We expected that the additional FGF, which has the ability to accumulate and stimulate fibroblasts at the surface of the PLAC cubes, would accelerate the generation process, but it did not. From the results we obtained, the reasons for this were unclear. However, the use of PLAC alone would be reasonable in human patients, as this would avoid possible progression of lung cancer due to FGF [22]. PLAC cubes are easy to manipulate. When a large dead space is expected after lung resection, application of PLAC cubes may be one option for possible obliteration of the dead space. Furthermore, the application of PLAC cubes in a sterile dead space can be done using video-assisted thoracic surgery.

In conclusion, the induction of fibrous tissue can be successfully achieved in the post-pneumonectomy dead space by implantation of PLAC cubes with or without gelatin microspheres releasing 100 μg of b-FGF. This safe and easy method may therefore be applicable in clinical situations after further investigation.

References