Prevention of late postpneumonectomy complications using a 3D printed lung in dog models

Xiaojun Li, Hui Cai, Xiaohai Cui, Peilong Cao, Jing Zhang, Gang Li and Jia Zhang

Second Department of Thoracic Surgery, The First Affiliated Hospital of Xi’an Jiaotong University, Xi’an, Shaanxi, China
Department of Anesthesia, The First Affiliated Hospital of Xi’an Jiaotong University, Xi’an, Shaanxi, China
Department of Pathology, The First Affiliated Hospital of Xi’an Jiaotong University, Xi’an, Shaanxi, China

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Abstract

OBJECTIVES: Repositioning of the mediastinum with implantation of a prosthesis seems the favoured approach to treat late complications of pneumonectomy caused by mediastinal shift. However, the traditional prostheses are not designed specifically for use in the thoracic cavity, sometimes resulting in failure of treatment for many reasons. The aim of our study was to develop a novel prosthesis to promote prevention or treatment of late postpneumonectomy complications.

METHODS: Using 3D printing technology, we created a novel mimetic lung model replicating the native one and then transplanted it into the thoracic cavity of postpneumonectomy dogs to maintain the original position of the mediastinum. Postoperative morbidity and mortality of late complications were compared between transplanted and non-transplanted groups. The safety and feasibility of implanting a 3D printed prosthesis were also evaluated by chest computed tomography (CT) scan and pathological examination.

RESULTS: At the 1-year follow-up, pneumonectomy dogs with 3D printed lungs showed less morbidity and mortality of late complications. CT images indicated dynamic mediastinal shift in pneumonectomy-only dogs with enlarged contralateral lungs. Nevertheless, there was no obvious change in the position of the mediastinum in 3D printed lung transplanted individuals. Moreover, the 3D printed lungs did not cause any additional side effects and revealed good histocompatibility and tolerance of recipients.

CONCLUSIONS: Our experiences indicated the safety, feasibility and efficacy of transplantation with 3D printed lungs for prevention of late postpneumonectomy complications and provided a practical and possibly unique clinical application of 3D printing technology for surgical therapy.

Keywords: Pneumonectomy • Complications • Prosthesis • Mediastinum • Postoperative care

INTRODUCTION

Pneumonectomy is a whole lung resection technique most frequently performed to treat central pulmonary cancer and for other indications including trauma, transplant and intractable end-stage tuberculosis or bronchiectasis [1–3]. The extremely high incidence of postoperative complications makes it a risky procedure [1]. Acute haemorrhage, thromboembolic disease, bronchopleural fistula (BPF), empyema and other respiratory cardiac complications occur in up to 40% of patients [4]. Most complications that occur early on are usually diagnosed in time and successfully treated. However, late complications that develop from a few months to many years after pneumonectomy are rare and more likely to be ignored, but can sometimes be life-threatening.

Many late postpneumonectomy complications have been reported over the past few decades, including late-onset chylothorax, tracheobronchomalacia, empyema, BPF and oesophagopleural fistula, among others [5]. In particular, postpneumonectomy syndrome (PPS) is the most common late complication in pneumonectomy patients, presenting with symptoms of dyspnoea, stridor, acute respiratory distress, dysphagia, heartburn, hoarseness, and recurrent bronchial and pleural infections [6, 7]. The classic aetiology of PPS is a shift of the mediastinum into the pneumonectomy space with clockwise or counterclockwise rotation, leading to compression of the tracheal bronchus, oesophagus, and the laryngeal nerve by either the aorta or vertebral column [8, 9]. A variety of treatment options have been described to manage PPS by repositioning of the mediastinum and the preferred approach is plombage of the pleural space with a saline-filled breast prosthesis or tissue expanders [7]. However, neither breast prostheses nor tissue expanders were designed specifically for use in the thoracic cavity. Their inappropriate size, tendency to migrate, and durability, alongside the risk of implant rupture, are reasons why this treatment can fail, with additional operative correction subsequently required [7, 10–12]. Therefore, novel implants that are specifically designed for use in the thorax are needed to avert these risk factors.

3D printing is a rapid prototyping technique that can make solid 3D objects, with any shape or structure, from digital models.
It has been widely used in tissue engineering [13, 14], pharmaceutical manufacturing [15] and cell research [16], and in assisting surgical planning [17, 18]. In this paper, we utilize this technique to create a mimetic lung model for implantation into the postpneumonectomy space of dogs and investigate the safety, feasibility and efficacy of this prosthesis.

MATERIALS AND METHODS

Animals and ethics

Forty-eight mongrel dogs, a mix of Greyhound, Labrador or Samoyed, were chosen with body weights ranging from 13 to 22 kg and randomly distributed in four groups: G1 (n = 12), left pneumonectomy group; G2 (n = 12), left transplant group that underwent left pneumonectomy and implantation of 3D printed prostheses; G3 (n = 12), right pneumonectomy group; G4 (n = 12), right transplant group that underwent right pneumonectomy and implantation of 3D printed prostheses.

This study was approved by the Institutional Research Committee of Xi'an Jiaotong University and performed at the Surgical Dream Works Laboratory. All dogs received humane care in compliance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health. The dogs were maintained on a standard light–dark cycle.

3D printing

We performed three steps to print 3D mimetic lung models: imaging of anatomical structures, digital 3D reconstruction and 3D printing. 3D images of lungs that would be excised were obtained from computed tomography (CT) of the chest and digitally reconstructed by computer software (Mimics 10.0, Materialise, Belgium). The subsequent digital files were generated and imported into a 3D printer (MEM-300-E, Tsinghua University, China) to produce 3D lung models. The material used for printing was polyethylene. The post-printing objects were then sterilized by irradiation and packed ready for intraoperative use.

Anaesthetic and surgical procedures

Following 12 h fasting and 6 h water deprivation, the dogs were anaesthetized by intravenous injection of 0.03 mg/kg pentobarbital (Merck, Germany), and then intubated with an endotracheal tube. Respiration was maintained by ventilating with supplemental oxygen and continuous O₂ monitoring. The saphenous veins were used to establish intravenous access for intra- and postoperative IV hydration. About 1.6 million units of benzyl penicillin sodium were given before and at the end of the operation to prevent perioperative infection. The following procedures were aseptic and carried out by the same surgical team, which consisted of one anaesthetist, one veterinary surgeon and one assistant. After skin preparation, the dogs were subjected to thoracotomy through the fifth intercostal space. Pneumonectomy was performed with the technique of individual ligation and cutting off pulmonary arteries, pulmonary veins and the main bronchus. Bronchus stumps were then sutured manually with 2-0 suture material after excision. For groups with implants, the 3D printed lungs were directly inserted into the postpneumonectomy cavity.

Before routine thoracic closure, a negative pressure chest drainage device (Worldgem Enterprise Development Co., China) was placed into the affected pleural cavity. All of the operations were conducted over 2 days in the following order: all G1 followed by all G2 on the first day; and all G3 followed by all G4 on the second day.

Postoperative care and data acquisition

Postoperatively, all animals received benzyl penicillin sodium by intravenous injection for 3 days, and were fed three times a day. Lactate ringer and hetastarch solutions were infused for postoperative fluid therapy. The negative pressure drainage devices were pulled out when the volume of drainage liquid was less than 30 ml. Skin sutures were removed after 10–14 days. All dogs were monitored for postpneumonectomy complications for a period of 1 year. Blood gas analysis of the dogs was carried out 3 months after operation. CT examination was performed every 3 months for a year, after which all dogs were sacrificed. Histological analysis was conducted on tissues from the diaphragm, pleura, pericardium and contralateral residual lungs.

Histological examination

Transverse sections of resected specimens were made and embedded in paraffin, and then stained with eosin and haematoxylin for morphological examination. Using an optical microscope, the presence of tissues was assessed and captured in each sample. Histological assessment of the slides was performed by two independent pathologists, blinded to the treatment modalities.

Arterial blood gas analysis

About 5 ml of blood was collected both before and 3 months after pneumonectomy, following anaesthesia. Blood gas measurements were made in duplicate and analysed on an automated gas analyser (Rapidlab 860, Siemens, Germany).

Statistical analysis

Results were expressed as mean ± standard deviation. Data were analysed by the paired or independent t-test between two paired samples or two independent groups. One-way ANOVA was used for within-group comparison. Fisher’s exact test was also used to compare variables and distributions relating to the dogs. Overall survival (OS) analyses were performed using Kaplan–Meier curves. The log-rank test was used to compare survivals between different groups. Data analysis was performed using IBM® SPSS Statistics (version 19.0). P-values less than 0.05 were considered significant.

RESULTS

3D printed lung models

3D images of lung were reconstructed and used for 3D printing. The mean time of manufacture for 3D lung models was 30.9 ± 5.8 h. The 3D printed lung models and the native lungs of the dogs, as presented in Fig. 1, had a very similar appearance. Table 1 presents...
the geometric characteristics of the 3D printed lungs compared with natural lungs. The liquid displacement method was used for measuring lung volume and showed that left 3D printed versus native lungs was $169.5 \pm 16.1$ vs $172.8 \pm 22.5$ ml, whereas right 3D printed versus native lungs was $393.9 \pm 52.6$ vs $406.8 \pm 52.6$ ml. A paired samples t-test revealed no significant difference in the volume, surface area, and weight between 3D printed and native lungs.

Subject demographic data

The demographic data for the study participants are summarized in Table 2. The mean age of the dogs was $3.4 \pm 0.7$ years, and the mean body weight was $17.9 \pm 2.6$ kg. One-way ANOVA analysis showed no statistical difference in age and body weight of these four groups. Although females predominated in each group, there was no significant difference between males and females. The mean surgical time was $52.0 \pm 12.3$ min for the two pneumonectomy groups (G1 and G3), and was $52.3 \pm 12.5$ min for the transplant groups (G2 and G4). Insertion of a prosthesis did not markedly prolong surgical time ($P = 0.926$). The amount of bleeding in each operative group was $12.4 \pm 4.7, 12.8 \pm 3.4, 12.7 \pm 3.9$ and $12.8 \pm 3.7$ ml, respectively. No significant difference was shown in these four operative groups.

Postpneumonectomy complications and outcomes

There was no acute morbidity among the dogs during or immediately after the operation. The negative pressure drains were pulled out 3–5 days after surgery. The mean volume of drainage liquid was $192 \pm 33$ ml. In total, 25% (12/48) of the dogs suffered from early complications, including one dog in G4 which suffered from incision infection simultaneously with contralateral pneumonia. No statistical difference was found between transplanted and non-transplanted groups (Table 3). Five dogs suffered from incision infection, and contralateral pneumonia occurred in four dogs within 1 week of surgery, which were cured by injection of benzyl penicillin sodium for a total of 7 days. One dog in G1 was found to have developed empyema by the 7th day postpneumonectomy. Additional antibiotic was used and the dog recovered 5 days later. BPFs that occurred in G2 and G3 dogs were restored by a repeated thoracotomy.

The total morbidity of late complications in the four groups was $12.5\%$ (6/48) and predominantly occurred in the non-transplanted groups (5/24 in non-transplanted groups vs 1/24 in transplanted groups). The major complication was PPS, which occurred more frequently in the right pneumonectomy group than in the left pneumonectomy group (Table 3). One dog in the G1 group suffered recurrent empyema that subsequently caused late-onset BPF. Most of the transplanted dogs did not show any adverse

<table>
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<th>Table 1: Geometric characteristics of 3D printed lungs and native lungs</th>
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<tr>
<td>Volume (ml)</td>
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<tr>
<td>Surface area (cm²)</td>
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<td>Weight (g)</td>
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<th>Table 2: Demographic data for participants</th>
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<tr>
<td>Age (years)</td>
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<tr>
<td>Mean</td>
</tr>
<tr>
<td>G1</td>
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complication caused by the prosthesis, except for one with chronic pleural cavity inflammation, which may have been induced by repeated friction between the prosthesis and pleura.

In total, six dogs died from late complications including five non-transplanted dogs that suffered from PPS (one in G1 and three in G3) and late-onset BPF (one in G1) and one transplanted dog (G4) from an uncharted disease. The survival curve is presented in Fig. 2. Dogs with 3D printed lung transplants showed improved survival compared with cases without transplantation, although the differences did not reach statistical significance (G1 vs G2, \( P = 0.148 \); G3 vs G4, \( P = 0.322 \)).

**Table 3: Complications of operative dogs**

<table>
<thead>
<tr>
<th></th>
<th>G1</th>
<th>G2</th>
<th>( P )-value</th>
<th>G3</th>
<th>G4</th>
<th>( P )-value</th>
</tr>
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<tbody>
<tr>
<td>Dogs with early complications (n)</td>
<td>3</td>
<td>3</td>
<td>1.000</td>
<td>3</td>
<td>3*</td>
<td>1.000</td>
</tr>
<tr>
<td>Infection of incision</td>
<td>0</td>
<td>1</td>
<td>1.000</td>
<td>1</td>
<td>3</td>
<td>0.590</td>
</tr>
<tr>
<td>Contralateral pneumonia</td>
<td>2</td>
<td>1</td>
<td>1.000</td>
<td>0</td>
<td>1</td>
<td>1.000</td>
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<tr>
<td>Empyema</td>
<td>1</td>
<td>0</td>
<td>1.000</td>
<td>0</td>
<td>0</td>
<td>1.000</td>
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<tr>
<td>BPF</td>
<td>0</td>
<td>0</td>
<td>1.000</td>
<td>0</td>
<td>0</td>
<td>0.478</td>
</tr>
<tr>
<td>Dogs with late complications (n)</td>
<td>2*</td>
<td>0</td>
<td>0.225</td>
<td>3</td>
<td>1</td>
<td>0.590</td>
</tr>
<tr>
<td>PPS</td>
<td>1</td>
<td>0</td>
<td>1.000</td>
<td>3</td>
<td>0</td>
<td>0.225</td>
</tr>
<tr>
<td>Late-onset BPF</td>
<td>1</td>
<td>0</td>
<td>1.000</td>
<td>0</td>
<td>0</td>
<td>1.000</td>
</tr>
<tr>
<td>Recurrent pleural cavity infection or empyema</td>
<td>1</td>
<td>0</td>
<td>1.000</td>
<td>1</td>
<td>1</td>
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*One dog suffered incision infection simultaneously with contralateral pneumonia.

**Arterial blood gas analysis**

The arterial blood gas of all dogs was analysed before and 3 months after pneumonectomy. The results demonstrated a marked reduction in arterial oxygen partial pressure (\( \text{PaO}_2 \)) post-right pneumonectomy (G3) when compared with preoperative values (78.4 ± 4.6 vs 90.6 ± 3.6 mmHg, \( P = 0.043 \)). As expected, the levels of \( \text{PaO}_2 \) in the right pneumonectomy dogs with 3D printed lungs (G4) were identical to preoperative values (86.3 ± 5.5 vs 88.9 ± 6.5 mmHg, \( P = 0.776 \)). However, in the left pneumonectomy groups, \( \text{PaO}_2 \) values did not significantly change after surgery either with (G2, 88.1 ± 7.2 vs 90.2 ± 4.7 mmHg, \( P = 0.624 \)) or without (G1, 85.5 ± 10.6 vs 89.7 ± 6.5 mmHg, \( P = 0.189 \)) 3D printed lung transplantation.

**Computed tomography and pathological examination**

CT scans were performed in each dog before and every 3 months after operation. Serial surveillance CT images indicated dynamic mediastinal shifts in both left and right pneumonectomy-only groups (G1 and G3) but no obvious changes were detected in the 3D printed lung transplant groups (G2 and G4). Typical chest CT images of dogs in each operative group are shown in Fig. 3. After pneumonectomy, the contralateral lungs progressively enlarged over the first 3 months and resulted in shift of the mediastinum (Fig. 3B and D). Compared with the pneumonectomy dogs, the lung volumes in the transplanted dogs were stable, reflecting the original position of the mediastinum (Fig. 3C and E).

To investigate the histocompatibility of the prostheses, all surviving dogs transplanted with 3D printed lungs were sacrificed 1 year after surgery. Macroscopically, the 3D printed lungs were stable in situ. Inflammatory adhesion was found between the prostheses and peripheral thoracic tissues, such as pleura and diaphragm (Fig. 4). Microscopic histological examination of the diaphragm, pleura and pericardium in operative hemithorax showed chronic inflammatory reactions around the prosthesis with fibroplastic proliferation and neutrophil infiltration (Fig. 5). Of note, slight chronic inflammatory reactions also existed in contralateral thoracic contents, especially in the residual lungs which presented with leucomonocytes surrounding the bronchus and hyperplasia of fibrous tissue and mesothelial cells (Fig. 5). No necrosis or other severe immunological rejection process was observed in the pathological tests.

**DISCUSSION**

At present, pneumonectomy is considered to be a risky procedure, due to the higher morbidity of postoperative complications which influence the quality of life and long-term survival [19].
Postpneumonectomy complications commonly occur within the 2 weeks following surgery and the appropriate management is usually provided immediately [3]. However, some complications may present several months, or even years, after the operation. These late postpneumonectomy complications are rare, but sometimes life-threatening. Among them, PPS due to shifting of the mediastinum is the most common [8]. In our study, PPS was identified in four postoperative dogs, one in the left pneumonectomy group (G1, 8.3%) and three in the right pneumonectomy group (G3, 16.7%). As in human beings, PPS is more likely to occur after right pneumonectomy because of the larger right lung volume, which results in a greater mediastinal shift [3]. After pneumonectomy, the residual cavity can lead to an over-expanded contralateral lung and mediastinal shift, in addition to other side effects, such as collapsed thorax and recurrent pleural cavity infection [20]. Our research showed that all pneumonectomy dogs in the G1 and G3 groups exhibited thorax collapse and two dogs had recurrent pleural cavity infection resulting in late-onset empyema and BPF.

To prevent the above complications, elimination of the dead space and repositioning of the mediastinum by the implantation of prostheses have been reviewed previously [5, 11, 21, 22]. Considering the low risk of migration, subsequent compression and erosion, tissue expanders and saline-filled breast implants are currently favoured for use [7]. However, the disadvantages of conventional breast prostheses or tissue expanders have also been identified [7]. The volume of the prosthesis is critical for maintaining mediastinal position [6]. In published reports, the volume of fluid that fills the prosthesis ranges from 120 to 1600 ml, with the most common volume approximately 1 l [7, 9, 11]. However, it is hard to accurately determine the instilled volume of fluid. Insufficient or excessive correction of mediastinal position may result in other symptoms due to compression of the remaining lung parenchyma [9, 11]. In this study, using 3D printing technology, we have successfully produced prototype models of dog lungs based on individual CT scans. Through a direct comparative validation, these models were shown to have a very high...
consistency with the native lungs in volume, surface area and weight. After insertion of 3D printed lung models in dogs, appropriate positioning of the mediastinum was achieved. Fluid-filled implants usually consist of a silicone shell that can sometimes be ruptured by mediastinal contents, such as a bronchial stump staple line, resulting in the prosthesis leaking and requiring replacement [7]. Moreover, Macaré van Maurik et al. [12] have reported implant migration behind the heart, resulting in dysphagia and expander herniation into the contralateral hemithorax, most likely due to deformation of the implant. Our 3D printed lung models are manufactured as solid objects made from polyethylene and have a fixed shape. After 1 year of follow-up, the 3D printed lung models were stable in vivo. None of the dogs in the G2 and G4 groups showed obvious shifting of the mediastinum and implant migration. Compared with the pneumonectomy-only groups, dogs with 3D printed lungs showed fewer complications and no noticeable collapse of the thorax.

3D printing technology is rapidly becoming an important clinical tool for the planning of complex surgery, and is considered superior to conventional 2D and 3D imaging for the visualization of anatomical structures with a real version that can provide greater intuitive navigation for critical anatomical landmarks [17, 18, 23, 24]. Here, we have presented another clinical usage of 3D printing technology: the production of biomimetic prostheses for postoperative transplantation. Although this mimetic lung model presents an improvement over traditional prostheses in terms of applicability and adaptability, certain limitations should be noted. Firstly, 3D printed models are based on CT or magnetic resonance images of native objects and, accordingly, will be prone to imaging errors [18]. According to the present study, most of the 3D printed lung models (18/24) were smaller than the native ones to varying degrees, although small margins of error are acceptable. Improvements in imaging resolution will increase the accuracy of these 3D printed models. Another potential limitation are the properties of the materials used. In this study, we utilized polyethylene as the 3D printing material as it has good histocompatibility, and no rejection reactions were observed in the dogs with implants. It is worth noting that the polyethylene-produced models were substantial and lacked plasticity. With respiratory movement, friction between the prosthesis and pleura may cause chronic inflammation and discomfort. Therefore, materials with lower friction coefficients or better pliability, such as polytetrafluoroethylene and silica gel, will be considered for future applications. Finally, the issue of cost is a constant concern when new technologies are being introduced [18]. The production of a 3D lung model for one dog took approximately 30 h, and the cost was about 500 US dollars including labour, materials and imaging. If this technology is eventually used in humans, the cost will be even higher. Thus, a number of initiatives are underway to improve the efficiency and lower the costs of production of 3D printed lung replicas.

In conclusion, we have presented the successful reproduction of canine lungs via 3D printing technology. These highly accurate models were transplanted into postpneumonectomy thoracic cavities to prevent mediastinal shifts and other late complications with higher efficiency and lower risk compared with conventional prostheses. This study, therefore, demonstrates practical and possibly unique clinical applications of 3D printing technology for surgical therapy.

Conflict of interest: none declared.

REFERENCES


