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Orthotopic Mouse Lung Transplantation as Experimental Methodology to Study Transplant and Tumor Biology

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Abstract

Unlike other solid organs, vascularized mouse lung transplantation has only recently been developed. In this publication we describe the detailed method for performing a vascularized and aerated mouse orthotopic lung transplant, which to date represents the most physiologic mouse model of lung transplantation. The procedure is divided into two separate portions consisting of donor harvest followed by implantation using the cuff technique for bronchovascular anastomoses. After a several month training period the procedure can be successfully mastered and in experienced hands requires approximately 90 minutes to perform. After an initial learning curve perioperative survival is close to 100%. As over time the donor hematopoietic cells in the transplanted lung are replaced by those of the host, thereby creating a “chimeric lung”, this model represents a novel research tool for the study of transplantation biology as well as other disease processes such as malignancies.

INTRODUCTION

Lung transplantation is the only treatment available for many patients suffering from end-stage pulmonary disease. While technical refinements, improvements in perioperative care, and advances in immunosuppression have led to acceptable short-term survival rates, long-term outcomes after lung transplantation are markedly worse than for other solid organs with five year survival rates of only 50%¹. Advances in long-term survival of other solid or cellular allografts have come through experimental manipulation of physiologically relevant mouse models, but no such models have existed for lungs².

The desire to study lung transplantation biology in the mouse led to the development of the heterotopic tracheal transplantation model, which was first described in 1993³.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

Transplantation of a tracheal segment into a subcutaneous pocket of an allogeneic recipient mouse is associated with progressive fibrotic occlusion of the airway lumen, a histologic feature resembling occlusion of small airways in human lungs undergoing chronic rejection. The technical simplicity of this model, the availability of multiple transgenic and knockout strains, and an extensive number of reagents such as monoclonal antibodies have led to its widespread use⁴. In fact, the heterotopic tracheal transplant model has been the most widely employed model for studying immunological aspects of lung transplantation and evaluation of immunosuppressive strategies for more than a decade⁵.

The importance of employing vascularized rather than nonvascularized transplant models has been already demonstrated for other organ systems. Nonvascularized grafts of neonatal cardiac tissue placed into a pouch in the ear pinna of a recipient mouse, for example, lack direct interaction with recipient blood and vascular endothelium of the graft. This model makes it difficult to draw conclusions about vascularized human cardiac grafts⁶. Similarly, the heterotopic tracheal model differs from human lung transplants in that it lacks vascularization and aeration. Another potentially important consideration is that the heterotopic tracheal transplantation model employs segments of large airway, while airway changes after human lung transplantation are observed in small airways. Furthermore, the rapidity of development of fibrotic occlusion of heterotopic tracheal allografts does not mimic observations in human lungs or vascularized lung grafts in large animals or rats⁷. We have also recently demonstrated that airway epithelial cells remain intact in acutely rejected mouse lung transplants despite extensive vascular necrosis, further documenting the nonphysiologic nature of the heterotopic tracheal transplant model⁸.

One of the unique features of lung grafts is that they are constantly exposed to the external environment. To this end, it has been demonstrated that the fate of human lung allografts can be regulated through exposure to agents that are introduced through the airway^{9,10}. Moreover, there has been recent interest in the inhalational administration of immunosuppressants¹¹. As the heterotopic tracheal transplant model is not aerated and therefore not exposed to the external environment, it does not allow for the design of studies to address these important issues in lung transplantation biology.

The basis for the development of the orthotopic mouse lung transplant model in our laboratory was the orthotopic single lung transplant model in the rat^{12,13}. In mice and rats, unlike humans, the left lung contains only one lobe and makes up only 25% of the total lung mass. This makes single left lung transplantation feasible in the murine model without the need for circulatory support. Since the initial development of this model we have established technical modifications of the model and now describe key steps that make the procedure easier to perform^{8,14,15}.

In addition to its obvious value for the study of transplant immunobiology, this model may also provide a powerful tool for dissecting pathogenetic contributions of pulmonary stromal cells to other disease processes. This is possible because after transplantation of a mutant syngeneic single lung near complete replacement of donor-derived hematopoietic cells occurs while the non-hematopoietic stromal cells remain of donor origin. One can thus create a “chimeric organ” without host irradiation or lung-specific expression of a transgene. The contralateral native lung may serve as an internal control within the same animal without global alteration of the host’s immune system.

MATERIALS

Reagents

The following list of reagents, microsurgical instruments as well as animal vendors represents our laboratory's preference. All instruments, reagents for anesthesia, and animal strains can be modified according to investigator preference unless indicated below.

- Male C57BL/6 and Balb/c mice 8–12 weeks of age (Jackson Laboratories, Bar Harbor, Maine, USA) (24–28grams)
- **CAUTION** Cuffed anastomoses in mice that are smaller than 24 grams are difficult to perform and mice over 28 grams in weight have too much fat in the pulmonary hilum for technically safe anastomoses
- **CAUTION** Experiments involving animals must be carried out according to institutional and national guidelines.
- Ketamine (50 mg/kg) (Wyeth, Madison, NJ, cat. No. 206205-01)
- Xylazine (10 mg/kg) (Lloyd Laboratories, Shenandoah, IA, cat. No. 139-236)
- Heparin solution (100 U/ml) (Abraxis Pharmaceutical Products, Schaumburg, IL, cat. No. 504031)
- Injection grade normal saline (0.9% sodium chloride) (Hospira Inc., Lake Forest, IL, cat. No. NDC 0409-4888-20)
- Clear chlorhexidine (Hibiclens, Mölnlycke Health Care US, LLC, Norcross, GA, cat. No. 57591)
- 70% Ethanol (Pharmco Products Inc., Brookfield, CT, cat. No. 111000140)

Equipment

- Microscope – Leica Wild M651 6–40× magnification, Leica Microsystems, Inc., Allendale, NJ
- ThermoCare Small Animal ICU System, (order from catalogue small ICS Unit with dome cover), Thermocare, Inc., Incline Village, NV (recovery settings 3L/minute O₂, 80°C, 40% humidity)
- Harvard Apparatus Mouse Ventilator Model 687 (Harvard Apparatus, Holliston, MA, cat. No. 550001) (settings 3L O₂/minute, respiratory rate 120 bpm, 0.5cc tidal volume)
- Ohio Isoflurane Vaporizer, (Parkland Scientific, Coral Springs, FL, cat. No. V3000i.)
- Bipolar coagulator, (Valleylab Inc., Boulder, CO., cat. No. SurgII-20, E6008/E6008B)
- Small animal retraction system (Fine Science Tools Inc., Foster City, CA, cat. No. 18200-20)
- Fine vannas style spring scissors (Fine Science Tools Inc., Foster City, CA, cat. No. 15000-03)
- Moria extra fine spring scissors (Fine Science Tools Inc., Foster City, CA, cat. No. 15396-00)
- 2 Dumont #5 forceps (Fine Science Tools Inc., Foster City, CA, cat. No. 11251-20)

- S&T SuperGrip Forceps straight and angled tip (Fine Science Tools Inc., Foster City, CA, cat. No. 00632-11 and 00649-11)
- 2 Halsted-Mosquito clamp curved tip (Fine Science Tools Inc., Foster City, CA, cat. No. 91309-12)
- Adson forceps (Fine Science Tools Inc., Foster City, CA, cat. No. 91127-12)
- Halsey needle holder (Fine Science Tools Inc., Foster City, CA, cat. No. 91201-13)
- Yasargil Temporary Aneurysm Clips (Aesculap, Inc., Center Valley, PA, cat. No. FT264T)
- Yasargil Clip Applier (Aesculap, Inc., Center Valley, PA, cat. No. FT480T)
- Perfadex™ low-potassium dextran glucose solution (Vitrolife Inc., Englewood, CO, cat. No. 19001)
- 6-0 Polydioxanone PDS II suture and (Ethicon Inc., Somerville, NJ. cat No. Z117H)
- 6-0 braided silk ties (Henry Schein Inc., Melville, NY, cat. No. 100-5597)
- 10-0 Nylon suture (Surgical Specialties Corporation, Reading PA cat. No. AK-0106)
- custom-made stabilization clamp used for stabilizing the vascular and bronchial cuffs during cuff placement (Figure 1)
- polystyrene petridishes, 150×25 mm and 60×25 mm (Becton Dickinson Labware, Franklin Lakes, NJ, cat. No. 353025 and 353004)
- 1/4 inch steel binder clip (Corporate Express, Broomfield, CO, cat. No. CEB35076)
- sterile Q-tips (tapered mini cotton tipped 3 inch applicators, cat. No. 823-WC, Puritan Medical Company LLC, Guilford, Maine)

Equipment for vascular and bronchial cuffs

- Surflew 20 gauge 1 1/4 inch Teflon angiocatheter (Terumo Medical Corporation, Elkton, MD, cat. No. SR-OX2032CA)
- Surflew 24 gauge 3/4 inch Teflon angiocatheter (Terumo Medical Corporation, Elkton, MD, cat. No. SR-OX2419CA)
- Surflew 20 gauge 1 inch Teflon angiocatheter (Terumo Medical Corporation, Elkton, MD, cat. No. SR-OX2025CA)

PROCEDURE

Preparation of Bronchial and Vascular Cuffs

TIMING 10 minutes for preparation of three cuffs

- 1 Prepare the bronchial and pulmonary venous cuffs from a 20 gauge Teflon angiocatheter 1mm in length, not counting the extension handle, using a # 11 scalpel with the metal needle of the angiocatheter as a cutting surface (Figure 2). Prepare the pulmonary artery cuff from a 24 gauge Teflon angiocatheter 0.6 mm in length in a similar fashion.
 - General Principles of Cuff Technique for Establishing Microvascular Anastomoses: Unlike larger vessels, which can be secured directly by suture, the microscopic bronchial and vascular anastomoses required

for mouse lung transplantation are performed using the cuff technique. The basic principles of this technique involve pulling the donor vessel through a rigid circular cuff sized to be slightly larger than the vessel (Figure 3A). The vessel is then folded over the cuff in order to expose the endothelial surface (or epithelial surface in case of the bronchus) and secured to the cuff with a 10-0 nylon tie (Figure 3B and C). This donor vessel is then inserted into the recipient vessel and the final anastomosis is secured with another 10-0 nylon tie (Figure 3D).

Donor Operation

TIMING 10–15 minutes

- 2 Anesthetize the donor mouse with an intraperitoneal (i.p.) injection of ketamine (50 mg/kg) and xylazine (10 mg/kg).
- 3 Place the anesthetized mouse under the operating microscope at 10× magnification in a supine position. After ensuring adequate anesthesia, cut down on trachea and place a tie of 6-0 silk suture around trachea.
- 4 Insert a 1 inch 20 gauge angiocatheter into trachea and connect to ventilator (Figure 4A).
- 5 Make a midline abdominal incision and incise diaphragm circumferentially. Place a Halsted-mosquito hemostat on diaphragm to retract it caudally and a similar hemostat on the xyphoid process to retract the sternum cranially, allowing for complete dissection of the diaphragm from the chest wall without damage to the lungs (Figure 4B).
- 6 Extend the incision cephalad to a midline sternotomy and place Halsted-mosquito hemostats on either side of sternum for retraction and hemostasis from the bone edges (Figure 4C).
- 7 Inject 0.1 cc heparin directly into the right lobe of the liver or the inferior vena cava.
CRITICAL Intrahepatic injection may result in less bleeding from the residual needle hole than an injection into the inferior vena cava.
- 8 Remove thymus and excise the left and right atrial appendage in order to vent any drainage from the lungs.
- 9 Inject 3 ml of cold 4°C Perfadex™ solution directly into the right ventricle using a 27 gauge needle on a 3cc syringe in order to flush all the blood from the lungs. It is important to maintain ventilation during flushing in order to ensure even distribution of preservation solution.
- 10 Clamp trachea proximally and then divide trachea distally. Dissect trachea caudally and posteriorly.
- 11 Remove heart-lung block and place in a small sterile petridish lined with gauze moistened with cold Perfadex™, which is placed into a larger petridish filled with ice (Figure 4D).
- 12 Place small gauze on left lung for retraction in order to expose left hilum (Figure 4E)
- 13 Carefully dissect the hilar structures from each other using angled forceps. Start by dissecting the pulmonary vein (PV) from the bronchus, located in the most caudal aspect of the hilum (Figure 4F). Next dissect the pulmonary artery (PA),

located in the most cranial aspect of the hilum from the bronchus in a similar fashion (Figure 4G).

- 14 Divide the PA away from hilum flush with the main PA in the mediastinum in order to ensure adequate length for placement of the cuff. Suspend the PA cuff approximately 3 mm above the pulmonary artery using the stabilization clamp and insert the PA inside the cuff (Figure 4H and 3A). Fold the PA over the cuff exposing the endothelial surface (Figure 3B and C). Secure around the cuff with 10-0 nylon (2 knots)(Figure 4I and 3B).

- 15 Divide PV away from hilum and place cuff on PV in identical fashion.

CRITICAL Do not divide bronchus at this stage. The bronchus is cuffed immediately prior to implantation in order to prevent entry of the preservation solution into the airway during storage. Place the lung block in 4°C Perfadex™ preservation solution.

Recipient Operation

TIMING 50–60 minutes

- 16 Anesthetize the donor mouse with an i.p. mixture of ketamine (50 mg/kg) and xylazine (10 mg/kg), shave the left chest and intubate. Intubation can be performed under direct vision by gently retracting the tongue out of the mouth and exposing the vocal cords with 10× magnification under the dissecting scope. The animal is intubated by inserting a 20 gauge 1 inch angiocatheter directly between the vocal cords (Figure 5A).

- 17 Place in right lateral decubitus position and clean with sterilization solution. It has been our preference to use clear chlorhexidine and 70% ethanol for chest wall preparation to prevent discoloration of the fur and skin (Figure 5B).

- 18 Incise the skin with scissors and perform the left thoracotomy completely with electrocautery.

CRITICAL Bovie electrocautery should be used for all portions of this dissection as it is important to minimize blood loss from the chest wall during this step.

- 19 Enter chest between ribs 3 and 4 and place 2 chest retractors (Figure 5C).

- 20 Place a sterilized 1/4 inch steel binder clip on recipient left lung and retract lung anteriorly to expose posterior hilum (Figure 5D). Dissect hilum bluntly using a sterile Q-tip.

- 21 Move clip posteriorly to expose anterior hilum for the rest of the dissection.

- 22 Dissect PA from bronchus using curved dissection forceps as far into the hilum as possible without tearing the vessel (Figure 5E).

- 23 Dissect bronchus from PV in a similar fashion (Figure 5F).

- 24 Place a one throw slipknot of 10-0 silk at base of PA and the PV (in that order) in order to occlude the blood flow to the left lung. Place an aneurysm microclip at base of bronchus to isolate the ventilation to left lung (Figure 5G).

- 25 Place 10-0 nylon around bronchus, PA and PV and leave loose (for future securing of cuffs).

- 26 Incise the recipient PA, bronchus, and PV close to the recipient left lung using microscissors and gently dilate the vascular and bronchial openings using curved

or straight forceps in order to create a large enough opening for placement of the donor cuffs.

- 27 Now cut the donor bronchus flush with the donor trachea and cuff the donor bronchus in a similar fashion to the PA and PV. Secure the cuff with 10-0 nylon (Figure 3D) and discard the donor right lung.
- 28 Place donor lung on top of retraction clip holding the recipient left lung and cover with small moist gauze.
CRITICAL The recipient left lung is left in place in order to put tension on the recipient hilum thereby facilitating the anastomosis.
- 29 Expose the cuffed hilar structures anteriorly and begin the anastomoses by inserting the donor into the recipient PA. The donor PA will be on some stretch at this point (Figure 5H).
- 30 After donor PA is inserted into recipient PA, place microclip across extension of the cuff to keep structures stabilized while securing the previously placed 10-0 nylon tie around recipient PA and cuff (2 knots)(Figure 5I and 3D). Remove the microclip from the extension cuff (Figure 5J).
- 31 Insert donor bronchus into recipient bronchus. The donor bronchus is on some stretch (Figure 5K).
- 32 After donor bronchus is inserted into recipient bronchus, place microclip across extension of cuff to keep structures stabilized while securing the previously placed nylon tie around recipient bronchus and cuff with 2 throws (Figure 5L and 3D). Remove the microclip from the extension of the bronchial cuff.
- 33 Insert donor PV into recipient PV with the donor PV slightly stretched (Figure 5M).
- 34 After donor PV is inserted into recipient PV, place microclip across extension of cuff to keep structures stabilized while securing nylon tie around recipient PV and cuff with 2 throws (Figure 5N and 3D).
- 35 Release slipknot on PV first followed by the PA to restore blood flow.
- 36 Next release the microclip on bronchus to restore ventilation.
- 37 Place the donor lung into chest cavity and remove recipient left lung by cutting remaining pieces of recipient PA, PV and bronchus posteriorly (Figure 5O). Discard the recipient left lung.
- 38 Close the chest with 6-0 PDS suture catching one rib above and one rib below the thoracotomy incision. Immediately prior to closure of the chest give the animal one large breath to evacuate any remaining air from the chest. Unlike in the rat model we do not put chest tubes into mice. Close the skin and subcutaneous tissue with a running subcuticular 6-0 PDS suture.

Animal Recovery

TIMING 12 hours

- 39 Allow the animal to recover with free access to food and water in the small animal ICU overnight.

ANTICIPATED RESULTS

Based on our experience with this model over the last 2 years we anticipate a learning curve of approximately 50 animals for any individual with basic microsurgical skills. Of note, one of the co-authors, who had no previous surgical training (X.L.), was able to master this technique by closely following this protocol. After an initial learning curve the perioperative mortality is near 0%.

SCIENTIFIC USES OF THE MODEL

Transplantation

We have already demonstrated that the transplantation of allografts in nonimmunosuppressed mice recreates histopathological findings observed in acutely rejected human lungs while syngeneic grafts remain free of inflammation⁸. Notably, alloimmune responses can be suppressed with perioperative blockade of costimulatory signals⁸. Various organs differ in their cellular requirements to initiate graft rejection and we have recently reported that, unlike cardiac grafts, the acute rejection of lungs is not dependent on CD4⁺ T lymphocytes^{15,16}. Extending the duration of cold ischemic storage is associated with a greater influx of neutrophils into the graft¹⁴. Therefore, this model may represent a more physiological tool in mice to study ischemia reperfusion injury after lung transplantation than the hilar clamping model that has been extensively utilized to date¹⁷. The Achilles heel of clinical lung transplantation remains bronchiolitis obliterans, which is defined as fibrotic occlusion of the small airways. Currently, heterotopic tracheal transplantation and graft versus host disease after bone marrow transplantation are models utilized to study airway changes that mimic human chronic rejection^{5,18}. However, we have recently shown marked differences in the fate of airway epithelial cells in non-immunosuppressed recipients of vascularized lung transplants compared to non-vascularized heterotopic tracheal transplants⁸ suggesting that observations made in heterotopic tracheal transplants need to be interpreted in the context of significant ischemic damage to the transplanted non-vascularized tissue. The vascularized lung transplant model will allow for the design of novel studies that examine the role of inflammatory stimuli such as respiratory viral infections or exposure to gastric acid on airway epithelial cells in allogeneic lung grafts. Future studies will need to determine whether vascularized lung transplants develop airway changes of chronic rejection after long-term immunosuppression.

Tumor Biology

One critical target for tumor rejection by T cells is the tumor stroma¹⁹. Experimental manipulation of tumor angiogenesis has proven to be a fruitful and rapidly growing field of therapeutic oncology²⁰. Experimental methods traditionally used to study tumor non-hematopoietic stromal cells have relied on the creation of bone-marrow chimeras by lethal irradiation and rescue with bone marrow of a mutant genotype. While widely utilized such methodology can create many confounding variables including graft versus host disease and autoimmune disorders due to altered development of T cells in a thymus where stromal and dendritic cells are of different genotype²¹. Lethal irradiation may also lead to unpredictable alteration in the physiology of irradiated antigen presenting cells and tumor vasculature^{22,23}. The ability to selectively vary the non-hematopoietic tumor stroma in one particular organ without lethal irradiation or alteration in any other tissue or organ systems may prove useful to the field of tumor angiogenesis as well as tumor immunology.

In order to investigate the utility of mouse lung transplantation specifically for studying the stromal component of tumors, we transplanted C57BL/6 MHC Class I deficient donors into C57BL/6 wild-type syngeneic recipients (B6I→B6) and used MHC Class I expression as a method for tracking donor and recipient cells. Such a system allows for validation of our

model without induction of an immune response to the transplanted lung as might occur with the use of other transgenic models carrying reporter genes such as GFP or β -galactosidase. Within 4 weeks of transplantation near complete replacement of the hematopoietic cells occurs in the recipient left lung while the non-hematopoietic cells remain of donor genotype (Figure 6). We have documented this level of replacement as long as 2 months post transplantation (data not shown).

In order to validate the lung transplantation model for the study of tumor biology we injected 2×10^5 B16 melanoma cells intravenously into six unmanipulated B6 mice in parallel to six sex- and age-matched B6 recipients of syngeneic B6 left lung isografts (Figure 7A,B). After 20 days, the native left lung of the mice without a transplant had an identical tumor burden compared to the transplanted B6 lung (Figure 7C). This suggests that the orthotopic lung transplant can support tumor growth in a similar fashion to the native lung further validating this model for the study of tumor biology.

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Figure 1. Custom made clamp for stabilization of cuffs

(A) We designed a custom made clamp from autoclavable materials, which allows for fixation of a needle holder or forceps (B) in order to facilitate the placement of vascular cuffs.

Figure 2. Creation of cuffs

Using an 11 blade (A+B) bronchial and vascular cuffs are fashioned with an extension cuff handle to facilitate placement (C+D)(PV=pulmonary vein, PA=pulmonary artery).

Figure 3. General Principles of Cuff Technique for Establishing Microvascular Anastomoses

The basic principles of cuff technique for microvascular anastomosis involve pulling the donor vessel through the circular cuff which is held in place by the extension handle of the cuff (Figure 3A). The vessel is folded over the cuff in order to expose the endothelial surface (Figure 3B) and secured to the cuff with a 10-0 nylon tie (Figure 3C). The cuffed donor vessel is then inserted into the recipient vessel and the final anastomosis is secured with another 10-0 nylon tie (Figure 3D).

Figure 4. Donor procedure

The donor operation proceeds with animal intubation via tracheostomy (A), dissection of the diaphragm (B) and a median sternotomy (C). The excised lung block is placed in a petri dish (D) and kept moist with gauze pads covering the lung (E). The hilum is dissected starting with the donor pulmonary vein (F), pulmonary artery (G) and all the structures except bronchus are cuffed (H,I).

Figure 5. Recipient procedure

The recipient operation begins with orotracheal intubation of the animal (A), right lateral decubitus positioning (B) and a left thoracotomy (C). The recipient left lung is freed from attachments in the posterior hilum (D) and the recipient pulmonary artery (E), bronchus and pulmonary vein (F) are dissected into the mediastinum. Proximal control of the recipient bronchus is obtained with an aneurysm clip and the recipient artery and vein are occluded with 10-0 silk (G). The cuffed donor pulmonary artery is inserted into the recipient pulmonary artery through an arteriotomy (H) and secured with a 10-0 nylon tie while the two are kept in close proximity with an aneurysm clip (I). The clip is removed from the pulmonary artery anastomosis but the tie occluding blood flow is left in place (J). The cuffed donor bronchus is then inserted into the recipient bronchus in a similar fashion (K) and secured in place with 10-0 nylon tie (L). The donor pulmonary vein (M) is inserted into the recipient vein in similar fashion (N). The lung is reperfed and placed back in the chest (O).

Figure 6. Replacement of hematopoietic cells in lung grafts

Using B6I- to B6 transplants we were able to determine that within one day of transplantation almost 40% of the hematopoietic cells are of recipient origin. Near complete replacement of the hematopoietic component of the left lung graft with recipient-derived cells is evident after one month, while the non-hematopoietic cells remain of donor origin (plots representative of three independent experiments).

Figure 7. Comparison of tumor burden between transplanted and native left lungs

Twenty days after injection of 2×10^5 B16 melanoma cells i.v. into six age and sex matched B6 mice as well as six B6 to B6 syngeneic transplant recipients the animals were sacrificed and the tumor burden was compared both visually (A,B) and by calculation of total tumor burden. There were no statistically significant differences in the tumor burden between the native and transplanted lungs (C).

TABLE 1**TROUBLESHOOTING**

Step	Problem	Reasons	Solution
15	Difficulty in PV cuffing	<ol style="list-style-type: none"> 1 The PV is cut too close to the left atrium 2 PV is not released enough. 	<ol style="list-style-type: none"> 1 Dissect the PV further into the left atrium 2 Release the PV gently from the bronchus all the way to its bifurcation
20	Poor exposure of the hilum	<ol style="list-style-type: none"> 1 Small incision 2 Chest entered in the wrong intercostal space 	<ol style="list-style-type: none"> 1 Enlarge incision 2 Cut the upper or lower rib
30	Recipient PA obstruction	<ol style="list-style-type: none"> 1 PA torsion (twist) 2 Air in the PA 3 Long PA cuff handle 	<ol style="list-style-type: none"> 1 Make sure the PA cuff is placed straight before insertion 2 Release the air and implant the PA again 3 Cut the long handle
33	Recipient PV obstruction	<ol style="list-style-type: none"> 1 PV torsion. 2 Thrombosis due to poor flushing of the graft 3 Long cuff handle 4 Compression from the bronchial cuff 	<ol style="list-style-type: none"> 1 Make sure the PV cuff is placed straight before insertion 2 Completely flush the donor lung until it is white 3 Cut the handle after implantation 4 Keep all the cuffs adjacent to each other without compression of vasculature
38	Pneumothorax	<ol style="list-style-type: none"> 1 Lung rupture 2 Bronchus rupture 	<ol style="list-style-type: none"> 1 Tie the hole if it close to the edge of the lung 2 Suture the bronchus with 11-0 nylon suture