Video Article

Mouse Pneumonectomy Model of Compensatory Lung Growth

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URL: https://www.jove.com/video/52294
DOI: doi:10.3791/52294

Keywords: Medicine, Issue 94, Pneumonectomy, Compensatory Lung Growth, Lung Injury, Lung Repair, Mouse Surgery, Alveolarization

Date Published: 12/17/2014

Citation: Liu, S., Cimprich, J., Varisco, B.M. Mouse Pneumonectomy Model of Compensatory Lung Growth. *J. Vis. Exp.* (94), e52294, doi:10.3791/52294 (2014).

Abstract

In humans, disrupted repair and remodeling of injured lung contributes to a host of acute and chronic lung disorders which may ultimately lead to disability or death. Injury-based animal models of lung repair and regeneration are limited by injury-specific responses making it difficult to differentiate changes related to the injury response and injury resolution from changes related to lung repair and lung regeneration. However, use of animal models to identify these repair and regeneration signaling pathways is critical to the development of new therapies aimed at improving pulmonary function following lung injury. The mouse pneumonectomy model utilizes compensatory lung growth to isolate those repair and regeneration signals in order to more clearly define mechanisms of alveolar re-septation. Here, we describe our technique for performing mouse pneumonectomy and sham pneumonectomy. This technique may be utilized in conjunction with lineage tracing or other transgenic mouse models to define molecular and cellular mechanism of lung repair and regeneration.

Video Link

The video component of this article can be found at https://www.jove.com/video/52294/

Introduction

The principal function of the lung is to provide for oxygen and carbon dioxide exchange between an organism and the atmosphere. In humans, a host of congenital and acquired conditions lead to reduced lung surface area which results in impaired lung function. Although a host of therapies such as inhaled corticosteroids, bronchodilators, supplemental oxygen, and chronic mechanical ventilation are used to mitigate the consequences of impaired lung function[1-3], the ideal therapy for these conditions would promote regrowth of functional lung tissue – *i.e.,* lung regeneration.

Mammalian tissue regeneration has been well documented. The African Spiny Mouse can regenerate large areas of skin without scar formation[4]. The distal phalanx in humans can regenerate following injury or amputation[5-7]. Following pneumonectomy (PNX), compensatory lung growth occurs in mice[8], rats[9], dogs[10], and humans[11]. By definition, compensatory lung growth involves not only expansion of existing airspaces, but re-septation of these enlarged airspaces with expansion of the associated microcirculation[12]. Gene expression analysis has demonstrated that this model recapitulates many of the signaling events of lung development[13]. Four weeks after mouse PNX, alveolar surface area is equivalent to that of sham operated animals[14]. In this manuscript, we describe the mouse PNX and sham PNX procedures.

Protocol

NOTE: Animal use statement: All procedures in this study were conducted with approval and following the guidelines of the Institutional Animal Use and Care Committee (IACUC) at Cincinnati Children's Hospital. Eight week-old C57BL/6J male mice were obtained from Jackson Laboratories (Bar Harbor, ME) and allowed to acclimate for one week prior to use. Up until surgery, animals were housed in a pathogen-free barrier facility and provided autoclaved chow and filtered water *ad libdium*. Each mouse cage was supplied with a dedicated air and water, and rooms were maintained on a 12 hr day-night cycle. Following recovery from surgery, mice were maintained in cages with filtered tops, provided autoclaved chow *ad libidum*, and provided filtered water from a water bottle.

1. Preparation of Instruments

1. Make 6 skin retractors using paper clips and pins. Twist straightened paper clips on the shanks of paper pins, leave a 5 cm straight steel wire on one end and make a single 0.5 cm “U” shaped hook at the end of the wire.
2. Make some 15 x 15 cm square surgical drapes using plastic wrap. Prepare one dressing per mouse. Put a paper tower in between each wrap.
3. Sterilize all surgical tools along with a stack of 12 x 12 inch cork tiles, gauze, and cotton-tipped swabs.

2. Mouse Preparation

1. Induce anesthesia with 2% isoflurane. Weigh animal.
2. In a dedicated surgical preparation area shave left thorax and neck area with electrical shaver.
3. Apply a drop of the artificial tear ointment to the mouse's eyes.
4. Decontaminate neck and left thorax with chlorhexidine and isopropyl alcohol. Repeat twice more.

3. Mouse Oro-tracheal Intubation and Mechanical Ventilation

1. Have a non-sterile surgical technician place the mouse supine in the pre-warmed surgical area.
2. Confirm depth of anesthesia by documenting lack of a response to paw pinch.
3. After washing hands and donning surgical attire, mask, and hat, don sterile surgical gloves.
4. After draping and using aseptic technique, make a 1 cm vertical incision over the anterior mid-neck to expose the larynx. Lightly retract the strap muscles with curved, serrated 10 cm forceps and expose the larynx and trachea by spreading the strap muscles with the tip of a straight scissors.
5. Orally insert a 22 G blunt-tip angiocatheter into the mid-trachea (**Figure 1A**) and visually confirm placement (**Figure 1B**). Maintain anesthesia and ventilate using 1-3% isoflurane through rodent ventilator (225 µl per stroke; 200 stokes per min). Employ a pressure limit of 15 cm H_2O.

4. Mouse Pneumonectomy

1. Lay the mouse in the right lateral decubitus position with the mouse's back facing the operator (left side up). Use a self-sealing plastic wrap as a sterile drape. Cutting through the drape, use blunt tipped curved scissors to make a 2 cm long cut parallel to the ribs at the 4^{th} and 5^{th} intercostal space. Insert the blunt tip curved scissors and dissect the skin away from the underlying ribs and intercostal muscles.
2. Retract the skin with four retractors to expose a square 1.5 x 1.5 cm surgical window (**Figure 2A**). Secure the retractors to the cork board.
3. Dissect down to ribs using curved forceps, and use one tip of the curved forceps to enter the thoracic cavity.
4. Using the blunt tip micro-scissors, use the lower blade to enter the thoracic cavity. Make a 0.5 cm incision between the ribs and repeat in the opposite direction.
5. Using the two remaining retractors, open the thorax in the anterior-posterior axis and the secure the retractors to the cork board (**Figure 2B**).
6. Using curved blunt-tipped forceps in the left hand, grasp the left lung and displace the upper portion of the left lung laterally and inferiorly through the thoracotomy until the left pulmonary artery and bronchus are exposed (**Figure 3A,B**).
7. Holding the loaded titanium vascular microclip applicator in the right hand with the body of the applicator in the palm and curved tip pointing away from the palm (**Figure 3C**), slide the applicator tip into the thorax along the curvature of the posterior aspect of the left lung and clip the left bronchus and pulmonary artery (**Figure 3D**).
8. Remove the applicator but keep the left lung retracted. Grasp the blunt tip micro-scissors with the right hand and cut the bronchus and pulmonary artery distal to the clip and remove left lung (**Figure 3E**).
9. Remove the rib retractors.
10. Use the curved blunt forceps to pinch up 1 cm of skin inferior to the incision but above the level of the diaphragm and insert a 24 G angiocatheter through the skin and into the left thoracic cavity (**Figure 4A,B**).
11. Use 5-0 prolene suture to place two interrupted sutures around the 4^{th} and 5^{th} ribs to close the thoracic cavity.
12. Remove the skin retractors. Use two sets of forceps to approximate the skin along the length of the incision and glue the skin closed.
13. Connect a 3 ml luer-lock syringe to the angiocatheter and remove residual air by applying gentle suction and withdrawing the angiocatheter.
14. Glue the neck incision closed using two sets of forceps as before.

5. Mouse Sham Pneumonectomy

1. Expose the left lung as noted in "Mouse Pneumonectomy" protocol. Lift the rib cage with curved blunt forceps to allow air into the left chest cavity (**Figure 5A,B**).
2. Place a 24 G angiocatheter into the left thoracic cavity as above being careful not to injure the left lung.
3. Using 5-0 prolene suture and being careful not to puncture the lung (**Figure 5C**), place two lengths of suture material into the $3^{rd}/4^{th}$ and $5^{th}/6^{th}$ rib interspaces (**Figure 5D**). Place both lengths of suture material before tying to lessen the risks of left lung herniation. Tie the suture material to make two interrupted stitches (**Figure 5E**).
4. Glue the skin over the thoracic incision, remove residual air with the angiocatheter, and glue the neck incision as above.

6. Resuscitation, Analgesia, and Recovery

1. Turn off the isoflurane, and administer 0.1 mg/kg of buprenorphine and 0.5 ml of normal saline subcutaneously.
2. When spontaneous respirations resume, remove the endotracheal tube.
3. Observe mouse until it is again ambulatory. Walking typically resumes several minutes after removal of the endotracheal tube.
4. Place the mouse in a 27 °C incubator (humidified, 25% oxygen) to recover O/N.
   NOTE: We place several pellets of chow wetted with water on the cage floor for the first 24 hr after surgery.
5. Administer 0.1 mg/kg of buprenorphine by intraperitoneal injection twice daily for three days after surgery. Take care not to open the surgical site when handling animals.

7. Mouse Monitoring

1. Weigh mice at 1, 3, 5, and 7 days after surgery.

Representative Results

A plot of PNX and sham operated mouse weights is provided in **Figure 6**. In our hands, survival is consistently 95 - 100% for both PNX and sham pneumonectomy. For descriptions of how the right lung re-grows in this model and the expected time course, we refer the reader to manuscripts of Gibney *et al.*[15] and Wang *et al.*[14]

Several common pitfalls must be avoided to successfully perform the mouse PNX and mouse sham pneumonectomy procedures.

Endotracheal Intubation: Many investigators learning the mouse PNX technique have difficulty with endotracheal intubation. As the mouse larynx is anterior and the endotracheal catheter must be passed through the vocal cords, it is easy for the catheter to pass into the esophagus. As demonstrated in **Figure 1A**, the blunt-tipped catheter should be angled as anterior as possible following the curvature of the tongue along the midline and then passed into the trachea with gently pressure. Mid-tracheal catheter position should be visually confirmed (**Figure 1B**). Endotracheal placement can be confirmed by gently moving the catheter side to side and observing for movement of the trachea. Proper positioning is confirmed by observing bilateral chest rise. An alternative technique is described below.

Surgical Field Creation: A second common pitfall is failure to create an adequate thoracotomy window. As illustrated in **Figure 2A** and **2B**, the thoracotomy window must be sufficiently large and posterior to allow adequate visualization of the left pulmonary artery and left main stem bronchus. The inexperienced operator will commonly make his thoracotomy too small, too anterior, or too inferior to easily retract the left lung and access the left hilum.

Vascular Clip Application: Failure to correctly apply the vascular clip will result in exsanguination. Proper identification of the hilum is critical (**Figure 3A**). As demonstrated in **Figure 3B** and **3C**, when the clip is removed from the holder by the clip applicator, the end of the clip is flush with the tip of the clip applicator and the body of the clip is within the body of the curved applicator tip. Gentle closure of the applicator handle will close the clip. Any alteration in clip orientation may result in inadequate clip closure; therefore, a new clip should be loaded if a clip becomes dislodged from the applicator. Likewise, traction on the left lung should be gentle so as not to tear the hilum proximal to the clip. The curved tip of the applicator should follow the curve of the superior aspect of the left lung to ligate the left hilum (**Figure 3D**). A common mistake is ligature of the esophagus. As demonstrated in **Figure 3E**, the esophagus lies just posterior to the hilum and can easily be ligated. Ligature of the esophagus should be suspected in mice that appear dehydrated or lose more weight than expected. An alternative ligature technique is described below.

Avoiding Left Lung Injury During Sham PNX: Probably the most common pitfall of experienced and inexperienced operators is left lung injury during sham PNX. As demonstrated in **Figure 5**, the operator must be careful during thoracotomy closure not to puncture the lung with the suture needle or to allow entrapment of herniated lung. It is important to lift the ribs when placing the retractor, the 24 G angiocatheter for air evacuation, and the suture (**Figure 5A-D**). During thoracotomy closure, placing both strands of suture before tying minimizes the risk of lung entrapment (**Figure 5E**). Our laboratory will commonly perform four sham procedures followed by four PNX so that we can convert a sham mouse into a PNX mouse should we injure the left lung. It should be noted that not all groups perform evacuation of residual left thoracic air. To the best of our knowledge, no data exists comparing PNX with and without residual air evacuation.

With practice and experience, both PNX and sham procedures should be accomplished in 10 min allowing for up to two dozen mouse surgeries to be performed in a single session.

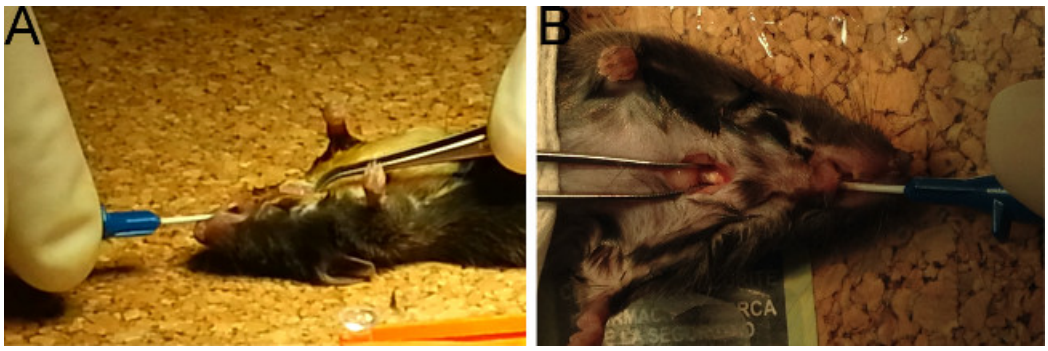

Figure 1. Mouse Endotracheal Intubation. (A) The oral-tracheal catheter should be midline and used to depress the tongue as it is advanced and angled anteriorly toward the larynx. **(B)** Proper positioning of the catheter in the mid-trachea should be visually confirmed. Please click here to view a larger version of this figure.

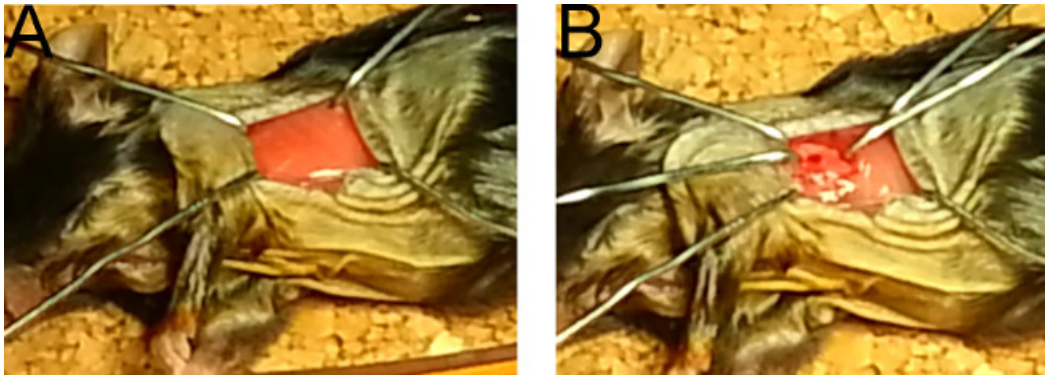

Figure 2. Mouse Thoracotomy. (A) After incision and release of the skin from the underlying thoracic cage, a 1.5 x 1.5 cm surgical window is created centered on the 4th and 5th intercostal space. **(B)** After creation of the thoracotomy in the 4th and 5th rib intercostal space, retractors are used to visualize the lung. Please click here to view a larger version of this figure.

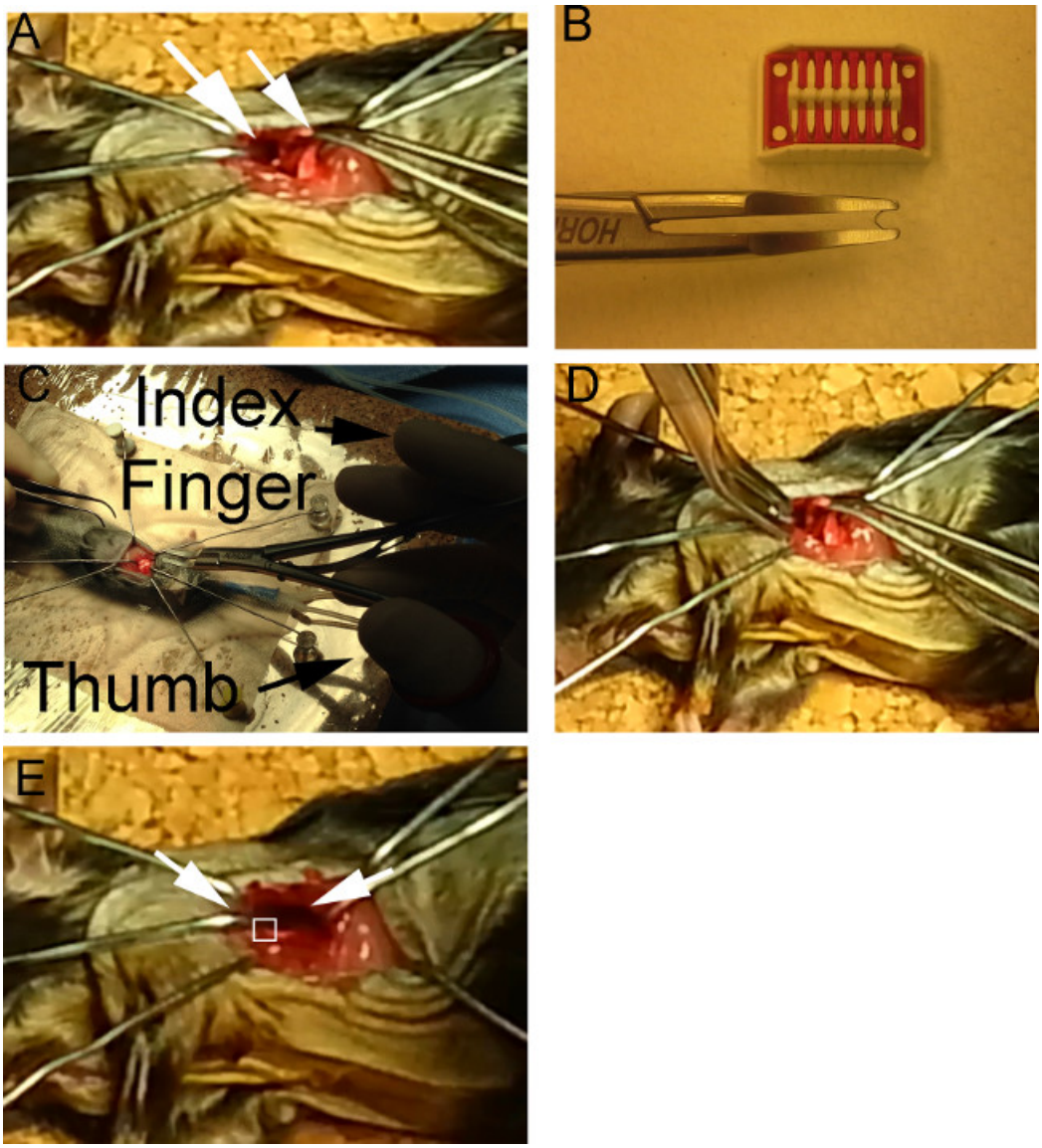

Figure 3. Pneumonectomy. (A) The left lung is grasped with curved forceps and lifted through the thoracotomy to reveal the left hilum (white arrow). **(B)** The vascular clip should sit flush with the tip of the vascular clip applicator and within the curved applicator tip. **(C)** The applicator should be held with the right thumb and 4th finger with the body of the applicator sitting along the palm of the right hand with the curved tip angled away from the palm. **(D)** Following the curve of the left upper lobe, the vascular clip is applied to the left hilum. **(E)** As seen following left PNX, the

esophagus (between the white arrows) is located just posterior to the left hilum (vascular clip outlined by white box). Please click here to view a larger version of this figure.

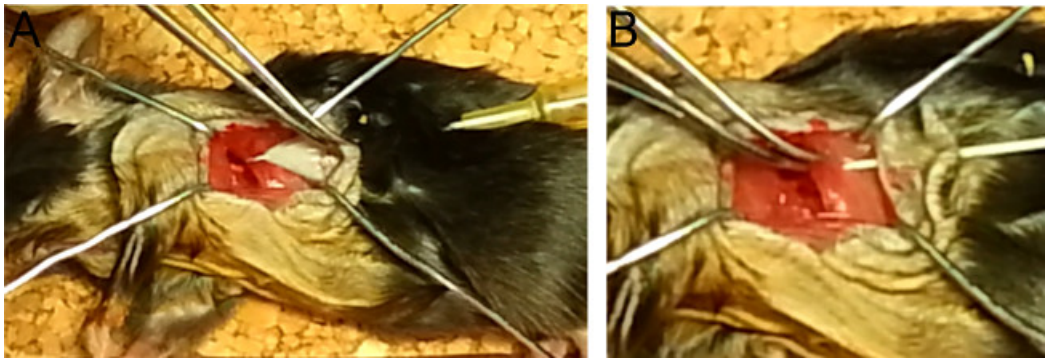

Figure 4. Placement of Angiocatheter for Air Evacuation. (A) The angiocatheter tip pierces the skin. **(B)** The angiocatheter is inserted above the diaphragm into the thoracic cavity. Please click here to view a larger version of this figure.

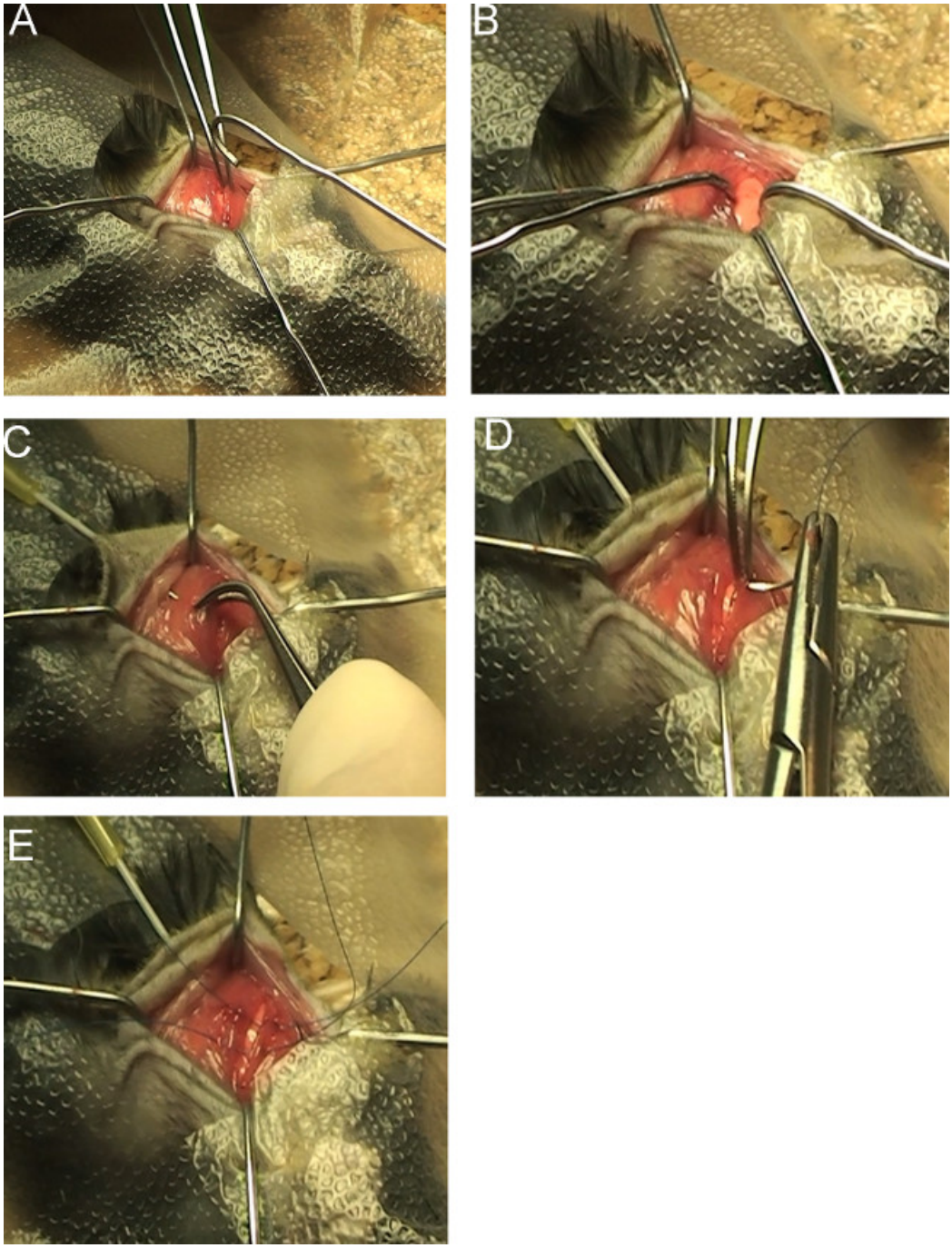

Figure 5. Mouse Sham pneumonectomy. (A) During the sham procedure, lifting of the rib edge prior to retractor placement minimizes risk of lung injury. **(B)** The retractors should be left in place as they are for the PNX. **(C)** The inferior edge of the rib cage should be lifted up when placing the 24 G angiocatheter used to aspirate residual air following closure of the thorax and overlying skin. **(D)** The rib cage should also be lifted for suture placement. **(E)** By placing two strands of suture over the thoracotomy site before tying, the risk of lung entrapment is minimized. Please click here to view a larger version of this figure.

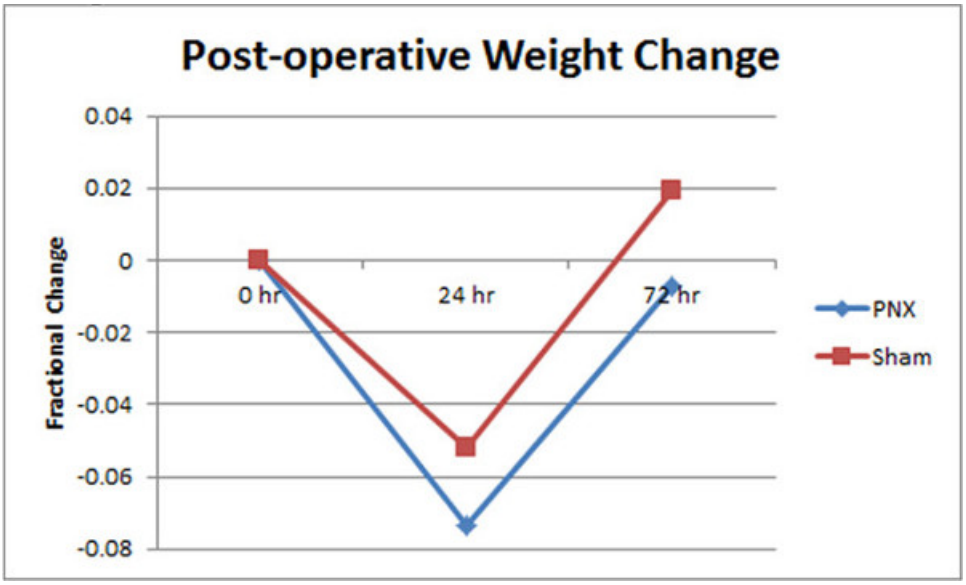

Figure 6. Expected Weight Loss. Using five mice per group, we demonstrated that 24 hr after surgery, PNX mice will lose an average of 1.5 g (7.2%), and sham mice will lose an average of 1.1 g (5.2%). Both groups regain their preoperative weight by postoperative day 3.

Discussion

We have provided the most detailed description of the mouse PNX and mouse sham PNX procedures reported to date. We have made the reader aware of several of the common pitfalls that investigators learning the procedure commonly encounter, and we have outlined several techniques developed by our laboratory to mitigate against these pitfalls. Other laboratories utilizing this model may have developed other technique modifications or use different instruments. When evaluating differences in techniques, individual investigators will need to decide whether or not these differences significantly impact experiment results.

The mouse PNX model of compensatory lung growth has many advantages over other animal models of lung repair and regeneration. The large numbers of transgenic mice available make lineage tracing, gene over-expression, and general of targeted gene deletion experiments achievable for most laboratories[16]. Second, morphometric techniques for quantitating alveolar re-growth are well-described[17]. Third, the cost of adopting this model is moderate. The only specialized equipment these procedures require is vascular clip applicators and blunt tip Cohan-Vannas spring scissors. All the other equipment will be commonly provided in a mouse surgical kit or available through a mouse surgical core facility. Fourth, with experience, the mouse PNX and sham PNX procedures are relatively quick allowing for many surgeries to be performed on the same day. Lastly, the small size of the mouse compared to other animal models, modest housing requirements, and the ease with which they can be bred also make the mouse model easier to maintain than other animal models.

There are also several disadvantages to the mouse PNX model when compared to other models of lung regeneration. While mouse compensatory lung growth is largely dependent upon stretch[18], compensatory lung growth in higher mammals is dependent upon both stretch and increased pulmonary blood flow[19]. Since human lung regeneration is likely more similar to the regenerative mechanisms of these higher mammals, the investigator should be careful extrapolating findings in the mouse directly to the human. Mouse respiratory mechanics are different than human respiratory mechanics. In humans, end exhalation is accompanied by a pause in which there is no airflow. At end exhalation in humans, lung recruitment is maintained by the radial traction of a low compliance thoracic cage. The mouse respiratory cycle lacks this expiratory pause resulting in a small amount of air trapping due to incomplete exhalation. Since the mouse has a much higher compliance thoracic cage, it is this air trapping which maintains lung recruitment[20]. The third disadvantage of the mouse model is its anatomy. Since the mouse has only a single left lung lobe, partial left PNX is not possible as it is in other species.

Several common pitfalls must be avoided at several critical junctures to successfully perform the mouse PNX and sham procedures. These critical junctures are endotracheal intubation, creation of the surgical field, vascular clip application, and avoidance of left lung injury during sham surgery.

Necropsy of any mice that fail to survive these surgical procedures is essential to improving surgical techniques in the future. Below, we troubleshoot several common difficulties and provide alternative techniques that may yield a greater degree of success.

Failure to Successfully Cannulate the Trachea: Other laboratories utilize suspension and trans-tracheal illumination for oro-tracheal intubation which obviates the need for direct visualization[21].

Difficulty Visualizing Hilum: Creating a large, properly placed thoracotomy window is necessary in order to visualize the left hilum. If during the procedure visualization is inadequate or there is insufficient space for insertion of the vascular clip applicators, the thoracotomy should be lengthened and retractors repositioned. There should be no pooling of blood during this procedure. If there is, the investigator should determine whether or not he is placing too much tension on the left lung and tearing the left pulmonary artery.

Bleeding After Removal of Left Lung: There should be little bleeding during this procedure. An improperly loaded or applied vascular clip is the most common cause of bleeding, and a mouse with bleeding from its left pulmonary artery should be euthanized. If this type of bleeding

occurs, proper loading of the vascular clip and the application of firm, even pressure during application should be confirmed before attempting the procedure again. Likewise, postoperative death with the finding of blood in the left chest is likely due to improper vascular clip application. An alternative to our vascular clip technique is ligature with silk suture.

Left Lung Injury During Sham Surgery: In many ways, the sham procedure requires a higher degree of surgical skill than the PNX procedure. The left lung can be injured during thoracotomy, during insertion of the 24 G angiocatheter below the surgical site, or during closure of the thoracotomy. Depending on the experimental need, mice undergoing sham surgery can be converted to PNX. Another potential cause of left lung injury is incarceration of herniated left lung during thoracotomy site closure. Sham mice with left lung injury should either be euthanized or have their surgical procedure changed to PNX.

Respiratory Distress After Recovery: Respiratory distress after recovery from anesthesia can come from swelling of the glottis due to traumatic intubation, inadequate pain control, or unrecognized lung injury. In general, unless the distress relieved by administration of additional pain medication, animals should be euthanized and cause determined. Multiple intubation attempts could result in glottic or subglottic swelling with resultant difficulty with inspiration. On necropsy, lack of a patent glottic opening will be apparent after the glottis is separated from the hypopharynx. Alternative approaches to endotracheal cannulation are described above. Inadequate pain control can be addressed with additional doses of pain medication. As noted above, unintended left lung injury is common when learning the sham surgical procedure, and if unrecognized, can lead to respiratory distress and alteration of experimental results.

Excessive Weight Loss and Dehydration: Mice that experience excessive weight loss or dehydration after surgery may have a ligated esophagus, inadequate pain control, or an inability to feed or drink. The esophagus lies just posterior to the left hilum, and ligation of the esophagus should be suspected in any dehydrated mouse. Inadequate pain control should be suspected in mice with piloerection, increased aggressiveness, or reduced ambulation. The thoracotomy can prevent mice from reaching food and water suspended from the cage roof on the first postoperative day. Mice that do not eat and drink after recovery from anesthesia will only become more weak and dehydrated. We recommend placing moistened food on the floor of the cage for 24 hr after surgery.

In conclusion, the mouse pneumonectomy model of compensatory lung growth is a valuable model for elucidating the underlying mechanisms of lung regeneration.

Disclosures

The authors have nothing to disclose.

Acknowledgements

The authors would like to acknowledge the Cincinnati Children's Hospital Division of Veterinary Services for their assistance. This manuscript was supported by the National Institutes of Health K12 HD028827. Anna Perl PhD taught the authors this surgical procedure.

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