Submission ID #: 61706
Scriptwriter Name: Bridget Colvin
Project Page Link: https://www.jove.com/account/file-uploader?src=18820378

Title: Evaluating Regional Pulmonary Deposition Using Patient-Specific 3D Printed Lung Models

Authors and Affiliations: Emma L. Peterman[1], Emily L. Kolewe[1], and Catherine A. Fromen[1]

[1]Department of Chemical and Biomolecular Engineering, University of Delaware

Corresponding Author:
Catherine A. Fromen
cfromen@udel.edu

Co-Authors:
emmap@udel.edu
ekolewe@udel.edu

Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or similar? **N**

2. **Software:** Does the part of your protocol being filmed demonstrate software usage? **N**

3. **Interview statements:** Considering the Covid-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interviewees wear masks until the videographer steps away (≥6 ft/2 m) and begins filming. The interviewee then removes the mask for line delivery only. When the shot is acquired, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. **Filming location:** Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Protocol Length
Number of Shots: **43**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Cathy Fromen**: This protocol has the potential to drive the development of new targeted pulmonary therapeutics by enabling the preclinical predictions of regional deposition **[1]**.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

REQUIRED:

1.2. **Emma Peterman**: This technique incorporates anatomically accurate lung models 3D-printed from patient CT scans for the rapid generation and delivery of personalized predictive results regarding the efficacy of potential treatments **[1]**.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

OPTIONAL:

1.3. **Cathy Fromen**: This technique can be used to develop targeted therapies that minimize off-target effects for diseases that are characterized by regional airway obstructions, such as lung cancer or COPD **[1]**.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Protocol

2. 3D Printed Experimental Component Preparation

NOTE: Sequence was shot 2.1.2., 2.2.1., 2.2.2., 2.1.3.

2.1. After printing the experimental components and completing post-processing as per the manufacturer's instructions **[1-TXT]**, carefully wash the parts printed in soft resin with at least 99%-purity isopropyl alcohol to remove any excess uncured resin **[2]** before thermal curing the parts in a convection oven for 8 hours according to the manufacturer's specifications **[3]**.

2.1.1. WIDE: Talent removing part(s) from printer **TEXT: See text for lung model part printing setup details**

2.1.2. Talent washing part(s), with isopropyl alcohol container visible in frame

2.1.3. Talent placing part(s) into oven

2.2. Then wash the parts printed in hard resin with the alcohol to remove excess any uncured resin **[1]** and cure the parts in a UV oven for 1 minute per side **[2-TXT]**.

2.2.1. Talent washing parts, with alcohol container visible in frame

2.2.2. Talent placing part(s) into oven **TEXT: Cure at 365 nm UV light at 5-10 mW/cm^2**

3. Lobe Outlet Cap Assembly

3.1. For lobe outlet cap assembly, insert one end of the oval barbed tubing connection base into the cap **[1]** before carefully stretching the flexible cap over the other end of the oval base, taking special care not to crack the thin base, and with the nozzle protruding through the opening in the cap base **[2-TXT]**.

3.1.1. WIDE: Talent inserting oval barbed tubing connection base end into cap

3.1.2. Cap being stretched over oval base, with nozzle protruding through opening in cap base visible in frame as possible **TEXT: Run two fingers along cap interior to stretch stiff, newly printed caps**

3.2. Next, cut 10-micrometer filter paper to a size slightly larger than the outlet area **[1]** and fold the filter paper over the lobe outlet, holding the paper in place with one hand **[2]**.

3.2.1. Talent cutting paper
3.2.2. Paper being stretched/held over lobe outlet

3.3. Then use tweezers in the other hand to stretch the cap with the barbed tubing connection over the outlet **[1]** and press the cap down until the notch matches the corresponding notch on the lobe outlet **[2-TXT]**.

3.3.1. Cap being stretched NOTE: Take 2 is just still shot showing cap after it has been fitted
3.3.2. Cap being pressed/notch being matched *Videographer: Important step* **TEXT: Repeat for all remaining lobe outlets** NOTE: Called "take two" twice instead of take one. In 2nd take, notch is indicated. Can be used if notch not visible in 1st take

4. Clinically Relevant Air Flow Profile Generation

4.1. Before each experimental run, connect each lung model lobe outlet to the tubing of the corresponding flow meter and valve, taking care not to apply too much lateral pressure to the barbed tubing connection **[1]**.

4.1.1. WIDE: Talent connecting outlet(s) to tubing *Videographer: Important/difficult step*

4.2. Attach the electronic flow meter to the lung model mouth inlet to measure the total air flow rate to the lung model **[1]** and turn on the flow controller and vacuum pump **[2]**.

4.2.1. Talent attaching flow meter
4.2.2. Talent turning on flow controller and/or vacuum pump

4.3. In the flow controller, select the **test setup** setting **[1]** and slowly increase the flow rate until the electronic flow meter displays the desired total flow rate **[2]**.

4.3.1. Talent selecting test setup setting, with monitor visible in frame
4.3.2. Flow rate being increased NOTE: Take 2 (file 6H0A2383) is actually shot 4.4.1.

4.4. Use the valves to adjust the flow rate through right upper, right middle, right lower, left upper, and left lower lung lobes **[1]**.

4.4.1. Talent adjusting valves *Videographer: Important step* NOTE: Take 2 for 4.3.2. (file 6H0A2383) is actually shot 4.4.1.

4.5. Once the lobe flow rates shown on the flow meters are steady at the desired value **[1]**, check the overall flow rate again on the electronic flow meter to verify that there are no leaks in the system **[2]**.

4.5.1. Shot of flow rate on at least one flow meter NOTE: file 6H0A2384 shot in 4k
4.5.2. Shot of flow rate on electronic flow meter NOTE: file 6H0A2385 shot in 4k

4.6. Then exit the **test setup** in the flow controller, leaving the vacuum pump on **[1]**.

4.6.1. Test setup being exited

5. Aerosol Delivery

5.1. For aerosol delivery to the lung model, fill a nebulizer with a solution of the desired fluorescent particles **[1]** and connect the nebulizer to the lung model inlet **[2]**.

5.1.1. WIDE: Talent filling nebulizer, with solution container visible in frame
5.1.2. Talent connecting nebulizer to inlet

5.2. To measure the efficacy of a targeting device, insert the device into the lung model **[1]** and connect the nebulizer to the device **[2]**.

5.2.2. Talent inserting device into lung model
5.2.1. Talent connecting nebulizer to device NOTE: Shot order switched from finalized script to order filmed as indicated here, otherwise same as original

5.3. Connect the compressed air line to the nebulizer **[1]** ~~and close the fume hood sash as much as possible **[2]**~~.

5.3.1. Talent connecting compressed air line to nebulizer
5.3.2. ~~Talent closing fume hood sash~~ NOTE: Shot of out order; should come after 5.5.1.

5.4. Set the flow controller to run for one 10-second trial **[1]** and open the compressed air valve slightly to begin generating an aerosol within the nebulizer **[2]**.

5.4.1. Talent setting flow controller
5.4.2. Talent opening compressed air valve

5.5. Press start on the flow controller and immediately open the compressed air valve fully. When the flow controller reaches about 9 seconds, begin closing the compressed air valve **[1]** and close the fume hood sash as much as possible **[2]**.

5.5.1. Talent pressing start, opening valve, then closing valve *Videographer: Important shot; can split action into separate shots if necessary* NOTE: Shot once with sash down (6H0A2407) but sash was dirty, so second take was filmed with under hood with sash up but close up

5.5.2. ADDED: Talent closing fume hood sash NOTE: Use 5.3.2., shot of out order; should be included here

5.6. Once the valve is fully closed, disconnect the nebulizer from the compressed air line **[1]**, fully close the fume hood sash **[2]**, shut off the vacuum pump **[3]**, and let any aerosols clear from the fume hood **[4]**.

5.6.1. Talent disconnecting nebulizer
5.6.2. Talent closing sash
5.6.3. Talent shutting off vacuum pump
5.6.4. Talent setting timer, with fume hood visible in frame

5.7. After about 10 minutes, disconnect the lung model from the tubing system, taking care not to crack the barbed tubing connections **[1]** and run a pair of tweezers under the edge of each lobe outlet cap to remove the caps from the outlets **[2]**.

5.7.1. Talent disconnecting model
5.7.2. Tweezers being used to remove cap

5.8. Then transfer the filter paper from each cap into individual wells of 24-well plate particle deposition-side down **[1]**.

5.8.1. Talent placing filter paper into well

6. Outlet Paper Imaging

6.1. When all of the filter paper has been collected, place the plate onto the stage of a digital fluorescence microscope **[1]** and set the microscope to a 4x magnification **[2]** and the appropriate fluorescence channel **[3]**.

6.1.1. WIDE: Talent placing plate onto stage
6.1.2. Talent selecting 4x magnification
6.1.3. Talent selecting channel NOTE: Shot filmed in 4k (files 6H0A2419 and 6H0A2420); file 6H0A2421 is 6.1.2. and 6.1.3. in one take, can use combined take or both individual takes

6.2. Then take at least three images of the filter paper from each lobe at random locations **[1]** and save the images as .tiff files **[2]**.

6.2.1. LAB MEDIA: Figure 3 High Deposition: Raw Image image
6.2.2. Talent saving file, with monitor visible in frame

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see?
3.3.2., 4.1.1., 4.4.1., 5.5.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success?
4.4.1. Both individual flow rates and the total flow rate are checked to identify if there are any leaks in the system. If flow rates are not in agreement, caps/filter paper can be replaced to make sure the system is airtight.

Results

7. Results: Representative Setup Validation and Targeting Experiment Data

7.1. Under 1 liter/minute collection conditions **[1]** in a healthy lung **[2]** and in a lung affected by COPD (C-O-P-D) **[3-TXT]**, the experimentally determined deposition profile is not statistically different from the clinical data **[4]**, demonstrating that the setup accurately mimics the distribution of air flow to each of the lung lobes **[5]**.

7.1.1. LAB MEDIA: Figure 4
7.1.2. LAB MEDIA: Figure 4 *Video Editor: please emphasize Figure 4A*
7.1.3. LAB MEDIA: Figure 4 *Video Editor: please emphasize Figure 4B* **TEXT: COPD: chronic obstructive pulmonary disease**
7.1.4. LAB MEDIA: Figure 4 *Video Editor: please add bracket and n.s. text over both graphs*
7.1.5. LAB MEDIA: Figure 4

7.2. Compared to the non-targeted particle deposition profile **[1]**, the use of a modified endotracheal tube **[2]** generates a nearly four-fold increase in left lower lobe delivery in addition to diverting over 96% of the delivered particles to the left lung **[3]**.

7.2.1. LAB MEDIA: Figures 5A-5C
7.2.2. LAB MEDIA: Figures 5A-5C *Video Editor: please emphasize schematic in Figure 5B*
7.2.3. LAB MEDIA: Figures 5A-5C *Video Editor: please emphasize light blue section of Figure 5A graph*

7.3. Altering the release location setting to target the right lower lobe **[1]**, this device generates more than double the particle delivery to the right lobe and diverts 94% of the delivered particles to the right lung **[2]**.

7.3.1. LAB MEDIA: Figures 5A-5C
7.3.2. LAB MEDIA: Figures 5A-5C *Video Editor: please emphasizes light green section of Figure 5C graph*

7.4. Compared to the non-targeted particle deposition profile **[1]**, the concentric cylinder device **[2]** causes a nearly three-fold increase in left upper lobe delivery in addition to diverting over 87% of the delivered particles to the left lung **[3]**.

7.4.1. LAB MEDIA: Figures 5D and 5E

7.4.2. LAB MEDIA: Figures 5D and 5E *Video Editor: please emphasize schematic in Figure 5E*

7.4.3. LAB MEDIA: Figures 5D and 5E *Video Editor: please emphasize dark blue section of Figure 5D*

7.5. Targeting efficiency can also be observed qualitatively by comparing the images of the target lobe filter to the other outlet filters **[1]**.

7.5.1. LAB MEDIA: Figure 3

7.6. As illustrated, the most effective targeting method will yield a high particle deposition at the intended lobe of interest **[1]** and a low deposition at the remaining lobe outlets **[2]**.

7.6.1. LAB MEDIA: Figure 3 *Video Editor: please emphasize High Deposition image row*

7.6.2. LAB MEDIA: Figure 3 *Video Editor: please emphasize Low Deposition image row*

Conclusion

8. Conclusion Interview Statements

8.1. **Emma Peterman**: It is essential to make sure that the components are properly connected to prevent leaks within the system, as leaks will impact the deposition results **[1]**.

8.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (3.3.2., 4.1.1.)

8.2. **Cathy Fromen**: This protocol permits researchers to test potential drug delivery devices for targeting specific regions of the lung prior to clinical trials, reducing the costs associated with drug development and efficacy enhancement in human patients **[1]**.

8.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera