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Title: Image-Guided In Vivo Tracking of Transplanted Distal Lung Epithelial Progenitor Cells for Pulmonary Fibrosis Using Magnetic Particle Imaging

Authors and Affiliations:

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Author Questionnaire

- 1. Microscopy:** Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **NO**

- 2. Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **NO**

- 3. Filming location:** Will the filming need to take place in multiple locations? **NO**

Current Protocol Length

Number of Steps: 19

Number of Shots: 31

Introduction

Videographer: *Obtain headshots for all authors available at the filming location.*

REQUIRED: ~~What is the scope of your research? What questions are you trying to answer?~~

- 1.1. **Saumya Nigam:** This study focuses on intratracheal intubation protocol as a critical delivery method for lung cell therapy, aiming to optimize cell transplantation and non-invasive tracking using magnetic particle imaging in pulmonary fibrosis.

- 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1*

What research gap are you addressing with your protocol?

- 1.2. **Saumya Nigam:** Our protocol addresses the lack of non-invasive methods for *in vivo* tracking of cell therapy in pulmonary fibrosis by combining intratracheal instillation with magnetic particle imaging to monitor cell distribution.

- 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.5.1*

What advantage does your protocol offer compared to other techniques?

- 1.3. **Saumya Nigam:** Our protocol offers precise, localized cell delivery via intratracheal instillation and superior tracking using magnetic particle imaging, providing high sensitivity, real-time imaging, with no background noise.

- 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.1.1*

How will your findings advance research in your field?

- 1.4. **Saumya Nigam:** Our findings will improve diagnostic accuracy and enable development of targeted therapies for pulmonary diseases, including stem cell therapy, accelerating progress and innovation in lung imaging and related biomedical fields.

- 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.2.1*

What research questions will your laboratory focus on in the future?

- 1.5. **Saumya Nigam:** Our laboratory will focus on augmenting stem cell-based therapies for lung fibrosis, enhancing non-invasive imaging techniques, while aiming to optimize cell delivery, and monitor therapeutic efficacy for pulmonary fibrosis.

- 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.3.1*

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the Michigan State University Institutional Animal Care and Use Committee (IACUC)

Protocol

2. Intratracheal Instillation of Bleomycin and Nanoparticle-Labelled Human Airway Epithelial Cells in Mice

Demonstrator: Saumya Nigam

2.1. To begin, weigh a 12-week-old male NOD or SCID (*nor or skid*) mouse to calculate the required dose of bleomycin [1].

2.1.1. WIDE: Talent placing the mouse on a digital scale.

2.2. Prepare the bleomycin solution using sterilized saline to a volume not exceeding 50 microliters in a 1.7-milliliter microcentrifuge tube [1] and load it into a pipette [2].

2.2.1. Talent pipetting sterilized saline and bleomycin into a microcentrifuge tube and mixing gently.

2.2.2. Talent aspirating the bleomycin solution into a pipette.

2.3. Preload a syringe with 200 microliters of air to ensure the entire liquid volume is expelled into the trachea while injecting [1].

2.3.1. Talent holding a syringe and pulling air into it.

NOTE: Show step 2.4 after step 2.13

2.4. Now, obtain nanoparticle-labelled human airway epithelial cells from lung explant samples of idiopathic pulmonary fibrosis patients undergoing lung transplantation [1]. Calculate the volume of cell suspension required to obtain 50,000 cells in a final volume of 50 microliters for administration [2]. After 72 hours of bleomycin administration, anesthetize the mouse again [3] and repeat the intratracheal intubation procedure to administer the cell suspension [4].

Added shot: Reuse 2.2.1 and 2.2.2. NOTE: We just need to show some tube with cells or pipetting clear solution to a tube. Reuse 2.2.1, but if the tube is labelled 'Bleomycin', then try to blur that label or use a different take where the label is not visible. This shot basically talks about pipetting cells. Contact poornimag@jove.com in case of doubts

2.4.1. Talent using a calculator and writing down the count in a notebook.

Added shot: Reuse 2.5.1 Positioning the anesthetized animal

Added shot: Reuse 2.10.2 Administering the cell suspension into the animal

- 2.5. After anesthetizing the mouse, assemble the fiber light intubation kit with the optical fiber probe threaded through the laryngoscope and catheter [1-TXT].
 - 2.5.1. Talent assembling the fiber light intubation kit on a lab bench, showing the probe correctly aligned through the laryngoscope and catheter. **TXT: Anesthesia: 2.5 - 3% Isoflurane**
- 2.6. To confirm the mouse is anesthetized, check the pedal reflex through a toe pinch on a hind leg [1] and suspend the mouse by its incisors in the supine position on an angled rodent intubation stand [2].
 - 2.6.1. Talent pinching the mouse's toe with forceps and observing the absence of reflex.
 - 2.6.2. Talent placing the anesthetized mouse in supine position with its incisors hooked on the intubation stand.
- 2.7. Using blunt-ended forceps, grasp the tongue and gently move it upward and leftward to expose the larynx for clear visualization [1].
 - 2.7.1. Talent carefully manipulating the mouse's tongue with blunt-ended forceps to reveal the larynx.
- 2.8. Turn on the fiber-optic probe and guide it into the mouth to approach the trachea [1]. Use the magnifier of the laryngoscope to enhance visibility and identify the vocal cords at the tracheal opening [2].
 - 2.8.1. Talent powering on the fiber-optic probe and inserting it through the mouth.
 - 2.8.2. Talent operating the laryngoscope.
- 2.9. Insert the catheter into the tracheal opening [1] and gently remove the laryngoscope [2]. Then, place a 20-microliter droplet of saline at the upper end of the catheter [3].
 - 2.9.1. Talent inserting the catheter into tracheal opening.
 - 2.9.2. Talent smoothly detaching the laryngoscope.
 - 2.9.3. Talent adding a small droplet of saline to the catheter tip.

2.10. Now, attach the syringe preloaded with 200 microliters of air and push the droplet into the trachea [1]. Load the catheter with up to 50 microliters of bleomycin and instill it using the air-filled syringe [2].

2.10.1. Talent connecting the air-loaded syringe and pushing the droplet into the trachea.

2.10.2. Talent instilling the bleomycin cell-suspension using the same syringe.

2.11. Keep the catheter in place for 5 to 7 seconds to prevent backflow of the liquid from the trachea [1]. Then, gently remove the catheter [2].

2.11.1. Shot of holding the catheter steady.

2.11.2. Talent slowly and carefully withdrawing the catheter.

2.12. Maintain the mouse on the intubation stand in the same position for at least 30 seconds [1].

2.12.1. Shot of the mouse on the stand.

2.13. Then, remove the mouse from the stand [1] and place it in a recovery cage positioned on a heating pad [2]. Monitor the mouse continuously until it fully recovers from anesthesia [3].

2.13.1. Talent removing the mouse from the stand.

2.13.2. Talent placing the mouse in the recovery cage placed on a heating pad.

2.13.3. Shot of the mouse showing signs of recovery.

NOTE: Show step 2.4 here after step 2.13

3. Magnetic Particle Imaging for Tracking Nanoparticle-Labelled Cells in the Mouse

3.1. Set up the mouse bed in the magnetic particle imaging scanner after passing the anesthesia line underneath the bed through the correct connectors [1].

3.1.1. Extra close-up of the mouse bed. *Videographer: Please try that the instrument*

manufacturer's name is not in the frame

- 3.2. While the mouse is being sedated, prepare the magnetic particle imaging scanner for imaging [1].
 - 3.2.1. Talent operating/turning on the magnetic particle imaging scanner system.
- 3.3. After confirming adequate anesthesia with a toe pinch test [1-TXT], carefully position the mouse on the magnetic particle imaging bed [2].
 - 3.3.1. Talent gently pinching the mouse's toe and mouse showing no reflex. **TXT: Anesthesia: 2 - 2.5% Isoflurane**
 - 3.3.2. Talent positioning the sedated mouse on the magnetic particle imaging bed.
- 3.4. Now, push the bed with the mouse into the scanner bore and click on **Start Scan** in the software interface to initiate imaging [1].
 - 3.4.1. Talent inserting the magnetic particle imaging bed into the scanner bore and initiating scan.
- 3.5. After the scan is completed, remove the mouse from the magnetic particle imaging bed [1] and place it into a recovery cage positioned on a heating pad [2].
 - 3.5.1. Talent gently lifting the mouse off the magnetic particle imaging bed.
 - 3.5.2. Talent placing the mouse into the recovery cage.
- 3.6. Finally, analyze the acquired images using image analysis software [1].
 - 3.6.1. Talent working at a computer looking at the acquired images.

Results

4. Results

- 4.1. The 2D magnetic particle imaging or MPI signal intensity increased proportionally with the concentration of the iron oxide tracer nanoparticle used for labelling across all tested cell counts [1], and the 250 micrograms per milliliter concentration was selected as the optimal labeling concentration [2].

4.1.1. LAB MEDIA: Figure 1A.

4.1.2. LAB MEDIA: Figure 1A. *Video editor: Highlight the blue bars labelled “250 µg/mL” across all cell counts*

- 4.2. Fluorescence microscopy confirmed successful in vitro labeling of cells, showing red Dextran signals in the cytoplasm and blue DAPI-stained nuclei [1].

4.2.1. LAB MEDIA: Figure 1B. *Video editor: Highlight the fluorescing coloured structures*

- 4.3. Signal intensities in 2D MPI images increased with the number of labeled cells, confirming a positive correlation between signal and cell count [1], and 50,000 cells were chosen for transplantation based on this trend [2].

4.3.1. LAB MEDIA: Figure 1C. *Video editor: Sequentially highlight the images from “10k” to “60k”*

4.3.2. LAB MEDIA: Figure 1C. *Video editor: Highlight the “50k” image*

- 4.4. The empty-bed control image showed no background MPI signal, confirming specificity of the scanner to labeled cells [1]. The four observed signals correspond to fiducial markers used as controls [2].

4.4.1. LAB MEDIA: Figure 3A.

4.4.2. LAB MEDIA: Figure 3A. *Highlight the 3 blue dots on top and one blue dot at the bottom*

- 4.5. Successful transplantation of labeled cells into the lungs produced a distinct butterfly-shaped MPI signal with visible distribution in both lungs, validating effective intratracheal delivery [2].

4.5.1. LAB MEDIA: Figure 3B. *Video editor: Highlight the symmetrical yellow signal*

4.6. Incomplete cell instillation led to signal appearing in both lungs and neck region, indicating leakage due to premature catheter removal [1].

4.6.1. LAB MEDIA: Figure 3C. *Video editor: Highlight both the bright yellow signals*

4.7. Misplacement of the catheter into the esophagus instead of the trachea caused the MPI signal to appear lower in the abdomen, indicating deposition of cells in the stomach [1].

4.7.1. LAB MEDIA: Figure 3D. *Video editor: Circle the bright yellow signal in the lower part of the image, below the lung area*

4.8. After 14 days, ex vivo imaging of the excised lungs revealed the retained presence of labeled cells, confirming transplant persistence [1].

4.8.1. LAB MEDIA: Figure 3E. *Video editor: Zoom in on the bright yellow signal in the middle on right panel and the lung tissue a the white platform towards the left*

1. Intratracheal

Pronunciation link: <https://www.merriam-webster.com/dictionary/intratracheal>

IPA: /,ɪn.trəˈtreɪ.ki.əl/

Phonetic Spelling: in·truh·tray·kee·uhl

2. Instillation

Pronunciation link: <https://www.merriam-webster.com/dictionary/instillation>

IPA: /,ɪn.stəˈleɪ.ʃən/

Phonetic Spelling: in·stuh·lay·shuhn

3. Bleomycin

Pronunciation link: <https://www.merriam-webster.com/dictionary/bleomycin>

IPA: /,bliː.ooˈmaɪ.sɪn/

Phonetic Spelling: blee·oh·my·sin

4. Nanoparticle

Pronunciation link: <https://www.merriam-webster.com/dictionary/nanoparticle>

IPA: /,næ.noʊˈpɑːr.tɪ.kəl/

Phonetic Spelling: nan·oh·par·tuh·kuhl

5. Epithelial

Pronunciation link: <https://www.merriam-webster.com/dictionary/epithelial>

IPA: /,ep.əˈθiː.li.əl/

Phonetic Spelling: ep·uh·thee·lee·uhl

6. Microcentrifuge

Pronunciation link: <https://www.merriam-webster.com/dictionary/microcentrifuge>

IPA: /,maɪ.kroʊˈsɛn.trəˌfjuːdʒ/

Phonetic Spelling: my·kroh·sen·truh·fyooj

7. Pipette
Pronunciation link: <https://www.merriam-webster.com/dictionary/pipette>
IPA: /paɪˈpet/
Phonetic Spelling: py·pet
8. Aspirating
Pronunciation link: <https://www.merriam-webster.com/dictionary/aspirate>
IPA: /ˈæs.pəˌreɪt/
Phonetic Spelling: as·puh·rayt
9. Syringe
Pronunciation link: <https://www.merriam-webster.com/dictionary/syringe>
IPA: /səˈrɪndʒ/
Phonetic Spelling: suh·rinj
10. Trachea
Pronunciation link: <https://www.merriam-webster.com/dictionary/trachea>
IPA: /ˈtreɪ.ki.ə/
Phonetic Spelling: tray·kee·uh
11. Idiopathic
Pronunciation link: <https://www.merriam-webster.com/dictionary/idiopathic>
IPA: /ˌɪ.di.əʊˈpæθ.ɪk/
Phonetic Spelling: id·ee·oh·path·ik
12. Pulmonary
Pronunciation link: <https://www.merriam-webster.com/dictionary/pulmonary>
IPA: /ˈpʊl.məˌner.i/
Phonetic Spelling: puul·muh·nair·ee
13. Fibrosis
Pronunciation link: <https://www.merriam-webster.com/dictionary/fibrosis>
IPA: /faɪˈbroʊ.sɪs/
Phonetic Spelling: fy·broh·suhs
14. Transplantation
Pronunciation link: <https://www.merriam-webster.com/dictionary/transplantation>
IPA: /ˌtræns.plænˈteɪ.ʃən/
Phonetic Spelling: trans·plan·tay·shuhn
15. Anesthetize
Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthetize>
IPA: /əˈnes.θəˌtaɪz/
Phonetic Spelling: uh·nes·thuh·tyze
16. Intubation
Pronunciation link: <https://www.merriam-webster.com/dictionary/intubation>
IPA: /ˌɪn.tuːˈbeɪ.ʃən/
Phonetic Spelling: in·too·bay·shuhn
17. Laryngoscope
Pronunciation link: <https://www.merriam-webster.com/dictionary/laryngoscope>
IPA: /ləˈrɪŋ.ɡoʊˌskoʊp/
Phonetic Spelling: luh·ring·goh·skohp

18. Catheter
Pronunciation link: <https://www.merriam-webster.com/dictionary/catheter>
IPA: /'kæθ.ə.t̬ər/
Phonetic Spelling: kath·uh·ter
19. Incisors
Pronunciation link: <https://www.merriam-webster.com/dictionary/incisor>
IPA: /ɪn'saɪ.zər/
Phonetic Spelling: in·sy·zer
20. Supine
Pronunciation link: <https://www.merriam-webster.com/dictionary/supine>
IPA: /'suː.pain/
Phonetic Spelling: soo·pyne
21. Larynx
Pronunciation link: <https://www.merriam-webster.com/dictionary/larynx>
IPA: /'ler.ɪŋks/
Phonetic Spelling: lair·ingks
22. Fluorescence
Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescence>
IPA: /flʊ'res.əns/
Phonetic Spelling: floo·res·uhns
23. Microscopy
Pronunciation link: <https://www.merriam-webster.com/dictionary/microscopy>
IPA: /maɪ'krɑː.skə.pi/
Phonetic Spelling: my·krah·skuh·pee
24. Dextran
Pronunciation link: <https://www.merriam-webster.com/dictionary/dextran>
IPA: /'dɛks.træn/
Phonetic Spelling: deks·tran
25. Cytoplasm
Pronunciation link: <https://www.merriam-webster.com/dictionary/cytoplasm>
IPA: /'saɪ.tʊɹ.plæz.əm/
Phonetic Spelling: sy·toh·plaz·uhm
26. Nuclei
Pronunciation link: <https://www.merriam-webster.com/dictionary/nucleus>
IPA: /'nuː.kli.aɪ/
Phonetic Spelling: noo·klee·eye
27. Fiducial
Pronunciation link: <https://www.merriam-webster.com/dictionary/fiducial>
IPA: /fɪ'duː.ʃi.əl/
Phonetic Spelling: fih·doo·shee·uhl
28. Esophagus
Pronunciation link: <https://www.merriam-webster.com/dictionary/esophagus>
IPA: /ɪ'sɑː.fə.gəs/
Phonetic Spelling: uh·saa·fuh·guhs

