FINAL SCRIPT: APPROVED FOR FILMING

Submission ID #: 69131
Scriptwriter Name: Pallavi Sharma
Project Page Link: https://review.jove.com/account/file-uploader?src=21085003

Title: A Refined Aerosol-Based Intratracheal Bleomycin Delivery Method for Reproducible and Minimally Invasive Mouse Models of Pulmonary Fibrosis

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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? **YES**

   If **Yes**, how far apart are the locations? 200m

4. **Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **NO**

Current Protocol Length
Number of Steps: 13
Number of Shots: 25

Introduction

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

1.1. **Yunyi Zhou:** We aim to develop a reproducible, minimally invasive aerosol-based delivery model for inducing pulmonary fibrosis and improving preclinical drug evaluation.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 2.15*

What advantage does your protocol offer compared to other techniques?

1.2. **Yunyi Zhou:** Our model enables consistent and reliable drug delivery while reducing surgical trauma and improving reproducibility across experiments.

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

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

1.3. **Ru Li:** We plan to apply this model to test our unique therapy for pulmonary fibrosis and chronic lung injury.

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

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

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at the Animal Studies Committee of the Institute of Basic Medical Sciences of Chinese Academy of Medical Sciences

Protocol

2. Preparation of Bleomycin Solution and Aerosol-Based Intratracheal Bleomycin Delivery

Demonstrators: Yunyi Zhou, Ru Li

2.1. To begin, using a sterile 1-milliliter syringe, withdraw 3 milliliters of sterile 0.9 percent sodium chloride **[1]**. Then, inject sodium chloride into a vial containing 15 units of bleomycin hydrochloride **[2]**.

2.1.1. WIDE: Talent drawing 3 milliliters of sodium chloride into a sterile 1 milliliter syringe from a sterile container.

2.1.2. Talent injecting the sodium chloride into a vial labeled "Bleomycin Hydrochloride – 15 U".

2.2. Gently swirl the vial until the bleomycin powder is fully dissolved to obtain a 5 units per milliliter stock solution **[1]**.

2.2.1. Talent gently swirling the vial.

2.3. Aliquot the stock solution into sterile 1.5-milliliter microcentrifuge tubes **[1]**. Then, prepare working solutions of 3.75 units per milliliter and 2.5 units per milliliter by diluting the stock with sterile saline **[2]**.

2.3.1. Talent pipetting the stock solution into multiple labeled sterile 1.5 milliliter microcentrifuge tubes.

2.3.2. Talent preparing dilution series of 3.75 and 2.5 units per milliliter using sterile saline and labeling the tubes accordingly.

2.4. After anesthetizing the mouse, prepare the aerosol delivery apparatus, ensuring the nebulizing needle, nebulizing syringe, and dosing pillar are all present **[1-TXT]**.

2.4.1. Shot of the aerosol delivery apparatus. **TXT: Anesthesia: 2,2,2-tribromoethanol and 2-methyl-2-butanol in saline**

2.5. Submerge the aerosol delivery syringe in sterile saline **[1]**. Slowly aspirate the fluid, pause for 8 seconds **[2],** then rapidly expel the fluid **[3-TXT]**.

2.5.1. Talent submerging the aerosol syringe tip into a beaker of sterile saline.

2.5.2. Talent slowly aspirating saline.

2.5.3. Talent expels the fluid. **TXT: Repeat 3x to fill and flush the system**

2.6. Then, refill the syringe with sterile saline after the final expulsion **[1]**. Attach the aerosolizing needle and ensure that the junction is filled with saline **[2]**. Then, submerge the fully assembled needle in sterile saline **[3-TXT]**.

2.6.1. Talent drawing saline back into the syringe after completing the previous flush.

2.6.2. Talent attaching the aerosolizing needle to the syringe.

2.6.3. Talent lowering the connected syringe and needle into a saline container. **TXT: Repeat 3x to eliminate any air bubbles**

2.7. To prime the aerosolizing needle with the bleomycin working solution, aspirate and expel it three times with 8-second pauses between each cycle **[1]**.

2.7.1. Talent aspirating the bleomycin working solution into the aerosol needle, then expelling it.

2.8. Aspirate the bleomycin working solution into the syringe **[1-TXT]**. Insert a 25-microliter dosing pillar on top of the 50-microliter pillar to calibrate the total volume **[2]**. Expel any excess solution **[3]**.

2.8.1. Talent drawing up a slightly larger volume of bleomycin solution into the syringe than the required dose. **TXT: Aspirate slightly more than the required volume**

2.8.2. Talent placing a 25 microliter dosing pillar on top of the existing 50 microliter pillar.

2.8.3. Talent expelling excess liquid carefully to adjust the volume.

2.9. Then, place the anesthetized mouse on the intubation platform **[1]**. Hook the upper incisors to the wire loop and tape the limbs to stabilize the mouse in position **[2]**.

2.9.1. WIDE: Talent transferring the anesthetized mouse onto the intubation platform.

2.9.2. Talent using a wire loop to secure the upper incisors and taping down the limbs gently.

2.10. Using curved forceps, gently pull out the tongue **[1]**, insert a small animal laryngoscope to visualize and expose the glottis **[2]**.

2.10.1. Talent pulling out the tongue using curved forceps with a gentle motion.

2.10.2. Talent inserting the laryngoscope into the oral cavity and adjusting it to clearly expose the glottis.

2.11. Then, insert the aerosolizing needle vertically through the exposed glottis **[1]** and deliver the bleomycin aerosol rapidly into the trachea **[2]**.

2.11.1. Talent positioning and inserting the aerosol needle through the glottis opening with precision.

2.11.2. Talent pushing the syringe plunger to rapidly deliver the aerosol.

2.12. After that, place the mouse on a warming pad to maintain body temperature **[1]**. Observe the mouse continuously until it fully recovers from anesthesia **[2]**.

2.12.1. Talent laying the mouse on a pre-warmed recovery pad.

2.12.2. Wide shot of the talent observing the mouse during the recovery period with attention.

2.13. Perform a toe pinch to check for reflexes and confirm that the mouse is alive **[1]**.

2.13.1. Talent using forceps or fingers to gently pinch the mouse's toe and observing for a reflex response.

Results

3. Results

3.1. Hematoxylin and eosin staining revealed mild alveolar wall thickening and localized fibrosis in the 5 units per kilogram bleomycin group **[1],** while the 7.5 units per kilogram group showed extensive alveolar collapse and severe fibrotic lesions **[2].**

3.1.1. LAB MEDIA: Figure 2A (middle panel). *Video editor: Zoom in on the magnified lung tissue section under "BLM 5.0 U/kg"*

3.1.2. LAB MEDIA: Figure 2A (right panel). *Video editor: Zoom in on the magnified lung tissue section under "BLM 7.5 U/kg"*

3.2. The modified Ashcroft score quantification demonstrated a dose-dependent increase in fibrosis severity in bleomycin-treated mice compared to saline controls **[1].**

3.2.1. LAB MEDIA: Figure 2B. *Video editor: Highlight the rising trend from "Saline" to "BLM 7.5 U/kg".*

3.3. Masson's trichrome staining revealed increased collagen deposition in the 5 units per kilogram group **[1]** and extensive interstitial fibrosis in the 7.5 units per kilogram group **[2].**

3.3.1. LAB MEDIA: Figure 2C (middle panel). *Video editor: Zoom in on the blue-stained regions of lung tissue under "BLM 5.0 U/kg".*

3.3.2. LAB MEDIA: Figure 2C (right panel). *Video editor: Zoom in on the dense, dark blue areas of collagen in the "BLM 7.5 U/kg" sample.*

3.4. Quantification of collagen-positive areas confirmed a significant, dose-dependent increase in collagen deposition following bleomycin treatment **[1].**

3.4.1. LAB MEDIA: Figure 2D. *Video editor: Emphasize the increasing height of the bars from "Saline" to "BLM 7.5 U/kg".*

3.5. Immunohistochemical staining showed increased expression of COL1A1 (*Col-One-A-One)* protein in lungs of both bleomycin groups **[1],** with stronger and broader staining observed in the 7.5 units per kilogram group **[2].**

3.5.1. LAB MEDIA: Figure 2E (middle panel) and F . *Video editor: Highlight the brown-stained cells in the "BLM 5.0 U/kg" image and blue bar in 2F*

3.5.2. LAB MEDIA: Figure 2E (right panel) and F. *Video editor: Highlight the more* *extensive brown-stained areas in the "BLM 7.5 U/kg" image and red bar in 2F.*

3.6. Hydroxyproline content was significantly higher in both bleomycin-treated groups, confirming increased collagen accumulation in lung tissue **[1].**

3.6.1. LAB MEDIA: Figure 3A.

3.7. Static lung compliance was significantly reduced in a dose-dependent manner in the bleomycin-treated groups **[1].** Inspiratory capacity was significantly lower only in the 7.5 units per kilogram bleomycin group compared to saline **[2].**

3.7.1. LAB MEDIA: Figure 3C.

3.7.2. LAB MEDIA: Figure 3B

3.8. Respiratory system elastance increased significantly in the 7.5 units per kilogram group compared to saline **[1].** Respiratory system compliance was significantly decreased only in the 7.5 units per kilogram group **[2].**

3.8.1. LAB MEDIA: Figure 3D. *Video editor: Emphasize the red bar labeled "7.5 U/kg",*

3.8.2. LAB MEDIA: Figure 3E. *Video editor: Highlight the drop in the red bar compared to the saline and 5 U/kg bars.*

1. Bleomycin

Pronunciation link:
https://www.merriam-webster.com/dictionary/bleomycin
IPA: /ˌbliːoʊˈmaɪsɪn/
Phonetic Spelling: blee-oh-my-sin

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2. Hydrochloride

Pronunciation link:
https://www.merriam-webster.com/dictionary/hydrochloride
IPA: /ˌhaɪdroʊˈklɔːrˌaɪd/
Phonetic Spelling: hy-droh-klor-ide

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3. Microcentrifuge

Pronunciation link:
https://www.merriam-webster.com/dictionary/centrifuge
(Word is formed from "micro" + "centrifuge"; "centrifuge" link applies)
IPA: /ˈmaɪkroʊˌsɛntrəˌfjuːdʒ/
Phonetic Spelling: my-kroh-sen-truh-fyooj

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4. Tribromoethanol

Pronunciation link:
No confirmed link found
IPA: /ˌtraɪˌbroʊmoʊˈɛθəˌnɔːl/
Phonetic Spelling: try-broh-moh-eth-uh-nawl

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5. Laryngoscope

Pronunciation link:
https://www.merriam-webster.com/dictionary/laryngoscope
IPA: /ləˈrɪŋgəˌskoʊp/
Phonetic Spelling: luh-ring-guh-scope

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6. Glottis

Pronunciation link:
https://www.merriam-webster.com/dictionary/glottis
IPA: /ˈglɑːtɪs/
Phonetic Spelling: glah-tis

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7. Trachea

Pronunciation link:
https://www.merriam-webster.com/dictionary/trachea
IPA: /ˈtreɪkiə/
Phonetic Spelling: tray-kee-uh

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8. Hematoxylin

Pronunciation link:
https://www.merriam-webster.com/dictionary/hematoxylin
IPA: /ˌhiːməˈtɑːksələn/
Phonetic Spelling: hee-muh-tok-suh-lin

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9. Eosin

Pronunciation link:
https://www.merriam-webster.com/dictionary/eosin
IPA: /ˈiːəsɪn/
Phonetic Spelling: ee-uh-sin

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10. Alveolar

Pronunciation link:
https://www.merriam-webster.com/dictionary/alveolar
IPA: /ælˈviːələr/
Phonetic Spelling: al-vee-uh-lur

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11. Fibrosis

Pronunciation link:
https://www.merriam-webster.com/dictionary/fibrosis
IPA: /faɪˈbroʊsɪs/
Phonetic Spelling: fy-broh-sis

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12. Ashcroft

Pronunciation link:
No confirmed link found (proper noun, used in pathology scoring)
IPA: /ˈæʃkrɑːft/
Phonetic Spelling: ash-kroft

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13. Trichrome

Pronunciation link:
https://www.merriam-webster.com/dictionary/trichrome
IPA: /ˈtraɪˌkroʊm/
Phonetic Spelling: try-krohm

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14. Interstitial

Pronunciation link:
https://www.merriam-webster.com/dictionary/interstitial
IPA: /ˌɪntərˈstɪʃəl/
Phonetic Spelling: in-ter-stish-uhl

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15. Immunohistochemical

Pronunciation link:
https://www.merriam-webster.com/dictionary/immunohistochemical
IPA: /ˌɪmjənoʊˌhɪstoʊˈkɛmɪkəl/
Phonetic Spelling: im-yoo-noh-his-toh-keh-mi-kuhl

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16. Hydroxyproline

Pronunciation link:
https://www.merriam-webster.com/dictionary/hydroxyproline
IPA: /haɪˌdrɑːksiˈproʊˌliːn/
Phonetic Spelling: hi-drok-see-pro-leen

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17. Compliance

Pronunciation link:
https://www.merriam-webster.com/dictionary/compliance
IPA: /kəmˈplaɪəns/
Phonetic Spelling: kuhm-ply-uhns

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18. Elastance

Pronunciation link:
https://www.merriam-webster.com/dictionary/elastance
IPA: /ɪˈlæstəns/
Phonetic Spelling: ih-las-tuhns