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Title: Intratracheal Administration of Dry Powder Formulation in Mice

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Author Questionnaire

1. **Microscopy**: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **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? **Same floor different rooms**

Current Protocol Length

Number of Steps: 15
Number of Shots: 30

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Jenny Lam:** Intratracheal administration of dry powder is essential to evaluate the performance and biological activities of an inhaled powder formulation such as pulmonary absorption, bioavailability and therapeutic effects in pre-clinical animal models.

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

1.2. **Jenny Lam:** The intubation process is non-invasive and could deliver powder formulations to the mice safely and accurately. The custom-made dry powder insufflator is disposable, inexpensive and efficient in dispersing powder formulations. The insufflators could be used in evaluating different formulations on multiple mice in the same experiment without the risk of cross-contamination from residual powder.

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

OPTIONAL:

1.3. **Yingshan Qiu (Carol):** The intubation process is challenging for non-experienced researchers. The ability to visualize and aim the finest tip of the cannula at the opening of the trachea is crucial for correct insertion of the guiding cannula. This technique requires practice to minimize improper insertion.

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

Introduction of Demonstrator on Camera

1.4. **Jenny Lam:** My postdoc Carol and my PhD student Rachel will demonstrate the preparation and intubation procedure.

1.4.1. INTERVIEW: Author saying the above.

1.4.2. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera.

Ethics Title Card

1.5. Procedures involving animal subjects have been approved by the Committee on the Use of Live Animals for Teaching and Research (CULATR) at the University of Hong Kong.

Protocol

2. Fabrication of Dry Powder Insufflator and Loading of Dry Powder

2.1. To begin, neutralize the static charges of the dry powder and the 200 microliter non-filter round gel-loading pipette tip with an anti-static gun or a balance with deionizing function **[1]**.

2.1.1. Talent using an anti-static gun.

2.2. Prepare a weighing paper with an approximate size of 4 by 4 centimeters. Fold the paper in half diagonally and then unfold it **[1]**, then use it to weigh 1 to 2 milligrams of dry powder **[2]**.

2.2.1. Talent folding and unfolding the paper.

2.2.2. Talent weighing powder.

2.3. Fill a gel-loading pipette tip with powder through the wider opening **[1]** and tap it gently to pack the powder until it forms loose agglomerates near the narrow end of the tip. Avoid packing the powder too tightly as it may hamper dispersion **[2]**. *Videographer: This step is important!*

2.3.1. Talent filling a pipette tip with the powder.

2.3.2. Talent tapping the pipette.

2.4. Connect the powder-loaded tip to a 1-milliliter syringe through a three-way stopcock, holding the tip and syringe vertically during connection to prevent spillage of powder. The size of the syringe can be changed according to the volume of air used to disperse the powder **[1]**.

2.4.1. Talent connecting the tip to a syringe.

2.5. If administration is not performed immediately, use parafilm to seal the openings of the tip and store it temporarily under suitable conditions until administration **[1]**.

2.5.1. Talent sealing the pipette tip.

3. Intubation

3.1. After anesthetizing the 7 to 9-week old BALB/c *(pronounce 'balb-C')* mouse with ketamine and xylazine, place it on a Plexiglass platform mounted to a stand **[1]**.

3.1.1. Talent positioning the mouse on a platform.

3.1.2. Talent adjusting the height or angle of the platform. NOTE: Please move shot 3.1.2 after 3.2.2 (before 3.2.3)

3.2. Suspend the mouse by hooking its incisors on a nylon floss **[1]** and secure its position with a piece of tape or a rubber band **[2]**. Adjust the height and angle of the platform [3.1.2]. Insert the optical fiber into the guiding cannula with the tip of the fiber level with the opening of the cannula **[3]**, then turn on the LED torch to illuminate **[4]**.

3.2.1. Talent hooking the mouse's incisors on a nylon floss.

3.2.2. Talent securing the mouse's position with tape or a rubber band.

Move 3.1.2 here

3.2.3. Talent inserting the optical fiber into the cannula.

3.2.4. Talent turning on the LED.

3.3. Gently protrude the tongue of the mouse with a pair of forceps to expose its trachea **[1]**. Use the other hand to hold the guiding cannula with optical fiber and insert them through the oral cavity **[2]**. With the illumination from the optical fiber, the opening of the trachea can be visualized as an orifice between the vocal cords **[3]**. *Videographer: This step is important!*

3.3.1. Talent protruding the tongue of the mouse.

3.3.2. Talent inserting the cannula into oral cavity. *Videographer: Use the video that the authors uploaded to the project page as a reference: VID-20171017-WA0000.MP4*

3.3.3. Visible opening of the trachea.

3.4. Align the bevel of the guiding cannula towards the midline of the opening **[1]** and gently intubate it with the optical fiber into the trachea by aiming the finest tip of the cannula at the tracheal opening **[2]**. *Videographer: This step is difficult and important!*

3.4.1. Talent aligning the guiding cannula. NOTE: Step 3.3.2, 3.3.3 and 3.4.1 were taken by phone (Clip IMG_2374.MOV; Clip IMG_2378.MOV; Clip_IMG_2379.MOV). The videos are uploaded to the project page. We think the first 10 seconds of Clip_IMG_2379.MOV is usable, with explanation in a ppt. file

3.4.2. Talent intubating the mouse.

3.5. Upon intubation, swiftly remove the optical fiber and leave the guiding cannula inside the trachea **[1]**. Normal respiration should be observed **[2]**.

3.5.1. Talent removing the optical fiber.

3.5.2. Mouse breathing normally.

3.6. Hold the fine tip pipette at the opening of the guiding cannula and insufflate a small puff of air into the lung of the mouse. A slight inflation in the chest indicates proper intubation **[1]**. Remove the fine tip pipette prior to powder administration **[2]**. *Videographer: This step is important!*

3.6.1. Talent insufflating a small puff of air into the lung of the mouse.

3.6.2. Talent removing the pipette.

4. Powder Administration

4.1. Hold the powder loaded tip that is connected to the syringe, making sure that the airflow between the syringe and the tip is disconnected **[1]**. Pull the syringe plunger backward to withdraw 0.6 milliliters of air **[2]**.

4.1.1. Talent making sure that the airflow between the syringe and tip is disconnected.

4.1.2. Talent pulling the syringe plunger back.

4.2. Turn the valve of the three-way stopcock to connect the airflow between the syringe and the powder-loaded tip **[1]**, then insert the powder-loaded tip into the guiding cannula which has already been placed in the trachea of the mouse **[2]**. *Videographer: This step is important!*

4.2.1. Talent turning the valve.

4.2.2. Talent inserting the tip into the cannula.

4.3. Hold the guiding cannula and push the syringe plunger forcefully in one continuous action to disperse the powder as aerosols into the lung, minimizing any forward motion to avoid injury to the animal **[1]**. *Videographer: This step is important!*

4.3.1. Talent pushing the syringe plunger in.

4.4. Remove the tip and check if the powder has been emptied **[1]**. Once the administration is complete, remove the guiding cannula from the trachea **[2]**. Allow the mouse to recover by positioning it horizontally in a supine position with its tongue half protruded to avoid a blockade of the airways **[3]**.

4.4.1. Talent checking the tip.

4.4.2. Talent removing the guiding cannula.

4.4.3. Mouse recovering.

Results

5. Results: Optimization of Intratracheal Administration of mRNA Formulation

5.1. To optimize the method, different volumes of air were used to disperse 1 milligram of the spray dried mannitol **[1]** and the weight of mice was monitored **[2]**.

5.1.1. LAB MEDIA: Figure 6. *Video Editor: Emphasize the 3 different volumes in the legend.*

5.1.2. LAB MEDIA: Figure 6.

5.2. The use of 0.3 and 0.6 milliliters of air did not cause weight loss of the mice up to 48 hours post-administration **[1]**. Dispersing the powder with 1 milliliter of air resulted in over 5% weight loss within 24 hours, which was not fully recovered after 48 hours **[2]**.

5.2.1. LAB MEDIA: Figure 6. *Video Editor: Emphasize the black and orange lines.*

5.2.2. LAB MEDIA: Figure 6. *Video Editor: Emphasize the blue line*.

5.3. The mice were intratracheally administered with 1 milligram of SFD powder containing 5 micrograms of mRNA and the luciferase expression in the lungs was evaluated at 24 hours post-administration using an in vivo imaging system **[1]**.

5.3.1. LAB MEDIA: Figure 7 A.

5.4. The SFD powder was dispersed in the deep lung and luciferase expression was observed **[1]**. As a comparison, the powder was reconstituted in water and administered with a microsprayer using the same intubation procedure **[2]**.

5.4.1. LAB MEDIA: Figure 7 A. *Video Editor: Emphasize the SFD powder image.*

5.4.2. LAB MEDIA: Figure 7 A. *Video Editor: Emphasize the SFD after reconstitution image.*

5.5. The luciferase expression of the reconstituted formulation was significantly higher than the dry powder formulation, which could be due to the powder dissolution issue or different pharmacokinetic profile between powder and liquid form **[1]**.

5.5.1. LAB MEDIA: Figure 7 B.

5.6. The histological characteristics of the lungs treated with mRNA dry powder aerosol were compared with untreated control and LPS-treated groups **[1-TXT]**.

5.6.1. LAB MEDIA: Figure 8. *Video Editor: Label A "***Control***", B "***LPS***", and C "***Dry Powder***".*

5.7. The lung without any treatment illustrated a healthy presentation **[1]** while the lung treated with LPS showed irregular distribution of air space and inflammatory cell infiltration into the interstitial and alveolar spaces **[2]**. The lungs treated with SFD powder did not show any signs of inflammation **[3]**.

5.7.1. LAB MEDIA: Figure 8. *Video Editor: Emphasize A.*

5.7.2. LAB MEDIA: Figure 8. *Video Editor: Emphasize B.*

5.7.3. LAB MEDIA: Figure 8. *Video Editor: Emphasize C.*

Conclusion

6. Conclusion Interview Statements

6.1. **Qiuying Liao (Rachel):** For successful powder dispersion, the powder in the loading tip should be tapped gently and not packed too tightly in the tip.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1 – 2.3.2.*

6.2. **Qiuying Liao (Rachel):** This intubation setting can also be adapted to administer liquid formulations, either with a pipette or microsprayer.

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