Submission ID #: 61793
Scriptwriter Name: Anastasia Gomez
Project Page Link: https://www.jove.com/account/file-uploader?src=18846913

Title: Cigarette Smoke Exposure in Mice using a Whole-Body Inhalation System

Authors and Affiliations:

Daniel E. Morales-Mantilla[1,2], Xinyan Huang[3,7], Phillip Erice[1,3], Paul Porter[4], Yun Zhang[1,5], Mary Figueroa[6], Joya Chandra[6], Katherine Y. King[2], Farrah Kheradmand[4,8], Antony Rodríguez[3,8]

[1]Program in Immunology, Baylor College of Medicine, Houston, TX, USA
[2]Department of Pediatrics, Section of Infectious Diseases, Baylor College of Medicine, Houston, TX, USA
[3]Department of Medicine – Immunology Allergy and Rheumatology, Baylor College of Medicine, Houston, TX, USA
[4]Department of Medicine, Pulmonary, Critical Care, Sleep Medicine, Baylor College of Medicine, Houston, TX, USA
[5]Department of Pathology and Immunology Baylor College of Medicine, Houston, TX, USA
[6]Department of Pediatrics, Research and Department of Epigenetics and Molecular Carcinogenesis, The University of Texas MD Anderson Cancer Center, Houston, TX, USA
[7]Division of Pulmonary and Critical Care Medicine, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong Province, China
[8]Center for Translational Research on Inflammatory Diseases (CTRID), Michael E. DeBakey VA Medical Center, Houston, Texas, USA

Corresponding Authors:

Antony Rodríguez (antonyr@bcm.edu)

Email Addresses for All Authors:

demorale@bcm.edu
xinyan.huang@bcm.edu
phillip.erice@bcm.edu
pp1@bcm.edu
yun.zhang@bcm.edu
mfigueroa1@mdanderson.org
jchandra@mdanderson.org
farrahk@bcm.edu

kyk@bcm.edu
antonyr@bcm.edu

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. **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 videographer steps away (≥6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, 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? **No**

Current Protocol Length

Number of Steps: 11
Number of Shots: 25

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Daniel E. Morales-Mantilla:** This technique can be used to study the physiologic effects of cigarette smoke on chronic airway inflammation and emphysema as well as bone marrow immune cell development in mice.

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

1.2. **Daniel E. Morales-Mantilla:** The low cost and the straightforward building procedure of the equipment makes it accessible to many labs around the world.

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

OPTIONAL:

~~1.3. **Daniel E. Morales-Mantilla:** While the technique can be used to study cigarette smoke-mediated emphysema and hematopoiesis in mice, it has the potential to be used for studying other organ systems impacted by cigarette smoke such as the gut and cardiovascular system.~~

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

~~1.4. **Daniel E. Morales-Mantilla:** The direction of the air flow and the connection of the components are critical for proper cigarette smoke administration to the animals. Some of the instructions may not be intuitive in a written format, and therefore visual demonstration of the technique will help the reader ensure that the machine is set up correctly with appropriate delivery of cigarette smoke and exposure of mice.~~

~~1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.~~

Ethics Title Card

1.5. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at Baylor College of Medicine and MD Anderson Institutions.

Protocol

2. Setting Pressure and Airflow

2.1. After assembling all components of the whole-body inhalation exposure system **[1]**, turn on the air compressor and wait for the safety alarm to turn off on its own **[2]**. *Videographer: This step is important!*

2.1.1. WIDE: Whole-body inhalation exposure system, with all components pre-assembled. *Videographer: Please make sure to get footage of the entire machine setup with the tubing and lines attached.*

2.1.2. Talent turning on the air compressor.

2.2. Adjust the pressure of the air compressor to 40 to 50 psi by turning the knob on the pressure regulator **[1]**. Adjust the airflow from the air compressor to 5 liters using the flowmeter **[2]**. Then, turn on the valve controller **[3]**. *Videographer: This step is important!*

2.2.1. Talent adjusting the pressure. NOTE: This and next shot together

2.2.2. Talent adjusting the airflow.

2.2.3. Talent turning on the valve controller.

2.3. Adjust the digital timer on the valve controller to the PULSE-C operating mode by pressing the SET-LOCK key while holding down the UP key at the first digit of the timer. Then, press the UP key until the Pu-c mode is reached. Press the RESET key to set the displayed operating mode as the working mode **[1]**.

2.3.1. Talent setting the mode on the timer. NOTE: This and next shot together

2.4. Press SET-LOCK to change timer 1, shown in the display as T1, then press the UP or DOWN keys to set it to 20 seconds. Repeat this process to set timer 2, or T2, to 3 seconds **[1]**.

2.4.1. Talent setting timers 1 and 2.

3. Cigarette Smoke Treatment

3.1. Turn on the air compressor and wait for the safety alarm to turn off on its own **[1]**, then turn on the valve controller **[2]**. Transfer 5 mice into each of the four 8.5-liter exposure chambers with airtight removable lids **[3-TXT]**.

3.1.1. Talent turning on the air compressor.

3.1.2. Talent turning on the valve controller.

3.1.3. Exposure chambers. **TEXT: Live animals were not used for this demonstration**

3.2. Place the exposure chambers inside a Class II *('2')* Type B2 Laminar Flow Biological Safety Cabinet **[1]**. Inside the biological safety cabinet, light a cigarette **[2]** and insert it into the cigarette chamber **[3]**. *Videographer: This step is difficult and important!*

3.2.1. Talent placing the exposure chambers into the biosafety cabinet.

3.2.2. Talent lighting a cigarette. NOTE: This and next shot together

3.2.3. Talent inserting the cigarette into the chamber.

3.3. Switch ON the valves on the valve controller that correspond to the chambers that are currently in use **[1]**. Clean air will be pumped into the exposure chamber for 20 seconds, causing the cigarette to burn, and smoke will be pumped into the exposure chamber for 3 seconds **[2]**. *Videographer: This step is important!*

3.3.1. Talent turning on the valves and pressing start.

3.3.2. Cigarette burning and smoke being pumped into the exposure chamber. *Videographer: Obtain multiple usable takes here, this will be reused in 3.6.1.*

3.4. Allow the cigarette to burn out completely until it reaches the filter. Adjust the timer settings to perform an average of 10 puffs per cigarette over a 4-minute period **[1]**.

3.4.1. Talent adjusting the timer setting. NOTE: Use end of 2.3.1

3.5. Remove the cigarette filter **[1]** and dispose of it by placing the cigarette butt in a glass beaker with water to extinguish the flame and dampen the odor **[2]**. Close the cigarette chamber and let the machine pump clean air for 10 minutes **[3]**. *Videographer: This step is important!*

3.5.1. Talent removing the cigarette filter and removing ash from cylinder. NOTE: 3.5.1 – 3.5.3 together

3.5.2. Talent putting the cigarette butt in the water beaker.

3.5.3. Talent closing the cigarette chamber.

3.6. Repeat the cigarette smoke exposure for a total of 4 cigarettes per chamber per day. When finished, remove the mice from the exposure chambers and place them back into their corresponding cages **[1]**.

3.6.1. *Use 3.3.2.*

3.7. Turn off the valve controller and the air compressor **[1]**. Remove the exposure and cigarette chambers **[2]** and wash them with water and soap to remove any residue of tar **[3]**. Let the chambers fully dry before using them again **[4]**.

3.7.1. Talent turning off the valve controller and air compressor.

3.7.2. Talent removing the cigarette chambers.

3.7a added shot: remove mouse

3.7.3. Talent washing the cigarette chambers.

3.7.4. Chambers drying.

Results

4. Results: CS-mediated Induction of Airway Inflammation and Lung Emphysematous Changes in Mice

4.1. Mice were exposed to the smoke of four commercial cigarettes daily, with smoke-free intervals of 10 minutes in between each cigarette, five days a week for a duration of 4 months **[1]**. Hematoxylin and eosin-stained lung histology showed the destruction of the alveoli in mice exposed to cigarette smoke **[2]**.

4.1.1. LAB MEDIA: Figure 2 A.

4.1.2. LAB MEDIA: Figure 2 A. *Video Editor: Emphasize the cigarette smoke image.*

4.2. Blinded histomorphometric analysis of lung sections confirmed that the mean linear intercept was significantly higher in mice exposed to cigarette smoke compared to air controls **[1]**.

4.2.1. LAB MEDIA: Figure 2 B.

4.3. As expected, whole-body exposure to cigarette smoke provokes a decrease in body weight **[1]**. The exposed mice also showed enhanced airway infiltration of immune cells **[2]** as well as induction of Matrix metalloproteases 9 and 12 gene expression, which are responsible for tissue damage **[3]**.

4.3.1. LAB MEDIA: Figure 2 C.

4.3.2. LAB MEDIA: Figure 2 D. *Video Editor: Emphasize the grey bars.*

4.3.3. LAB MEDIA: Figure 2 E. *Video Editor: Emphasize the grey bars.*

4.4. Cotinine, a metabolite of nicotine, was significantly elevated in the serum of mice exposed to 4 months of cigarette smoke **[1]** but undetectable in air-exposed mice **[2]**.

4.4.1. LAB MEDIA: Figure 2 F. *Video Editor: Emphasize the grey bar.*

4.4.2. LAB MEDIA: Figure 2 F. *Video Editor: Emphasize the absence of the Air bar.*

4.5. After exposure, bone marrow populations were analyzed using flow cytometry **[1]**. A significant increase in hematopoietic stem and progenitor populations after 4 months of cigarette smoke exposure was detected **[2]**.

4.5.1. LAB MEDIA: Figure 3 A.

4.5.2. LAB MEDIA: Figure 3 B. *Video Editor: Emphasize the HSPCs graph.*

4.6. Whole-body exposure to cigarette smoke of mice utilizing a commercially available system also showed an alteration in HSPC populations **[1]**.

4.6.1. LAB MEDIA: Figure 3 C. *Video Editor: Emphasize the HSPCs graph.*

Conclusion

5. Conclusion Interview Statements

5.1. **Daniel E. Morales-Mantilla:** It is important to ensure that all of the tubes delivering air and cigarette smoke to the mouse chambers are unobstructed and free of kinks that could disrupt airflow. Furthermore, mice need to be monitored at all times during exposure to cigarette smoke.

5.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.3.1, 3.3.2.*

5.2. **Daniel E. Morales-Mantilla:** The procedure can also be used to study the physiologic effects of cigarette smoke on other organ systems such as the heart and GI-tract.

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

5.3. **Daniel E. Morales-Mantilla:** With a few modifications, this machine can also be adapted for whole-body inhalation exposure of mice to electronic cigarettes. This could allow researchers to study the potential hazards associated with VAPING in a murine animal model.

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