Lung Tissue Micro-Structure Evaluation Following Chronic Cigarette Exposure

Northeastern University Center for STEM Education

YOUNG SCHOLARS PROGRAM

Katherine Klosterman, *YSP Student, Arlington High School*
Oliver Trejo, *YSP Student, Beaver Country Day School*
Jacqueline Matz, *Ph.D. Candidate, Northeastern University*
Dr. Jessica Oakes, *Bioengineering, Northeastern University*

NSF

Northeastern University College of Engineering

Abstract

The Integrated Cardiovascular and Pulmonary Team (ICAP), led by Professor Bellini and Professor Oakes in the Department of Bioengineering, is interested in the long-term health consequences following exposure to cigarette smoke and electronic cigarette (e-cig) aerosols. The goal of this project is to quantify cardiopulmonary dysfunction that occurs in mice as a result of chronic exposure. At the conclusion of this study, lung tissue was collected, fixed, and stained to assess and compare micro-structure. In this work, MOVAT staining was used to quantify bronchi thickness following chronic (six month) exposure to cigarettes.

Introduction

Almost 1 in 5 deaths in the United States are associated with cigarette smoking which amounts to 480,000 deaths each year caused by smoking. Chronic smoking is the primary cause of a number of cancers and diseases. This list includes chronic obstructive pulmonary disease (COPD), emphysema, chronic bronchitis, and lung cancer (2, 8). More symptoms include chronic cough, increased phlegm production, wheezing, and dyspnoea (5). Smoking is the leading preventable cause of death worldwide, yet it still claims millions of lives per year (5). In 2016, the World Health Organization (WHO) reported that COPD caused 3.0 million deaths and lung cancer caused 1.7 million deaths worldwide (Fig. 1)(9).

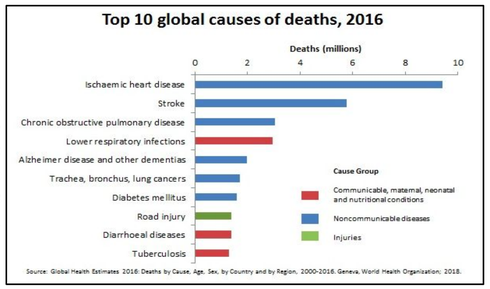


Figure 1: The top 10 global causes of death in 2016 according to WHO. Smoking is a leading cause of both COPD and lung cancer which are the third and sixth highest causes of death worldwide.

Smoking causes these diseases because it damages airways and alveoli in the lungs, which prevents proper airflow which makes it harder for patients to breathe (8). COPD is characterized by airflow obstruction and limitation, lung inflammation, emphysema, and mucus hypersecretion (3, 5). Another consequence of smoking that researchers have been looking into more recently is airway thickening. In one investigation, researchers concluded that airway wall thickening is a key marker of airway injury caused by smoking. This thickening is thought to be caused by changes induced by lung inflammation as well as airway remodeling (1). In this investigation, the effects of chronic cigarette exposure on airway wall thickness in mice will be explored.

Methods

ImageJ/Fiji was used in order to segment and crop histological images (Fig. 2A) of bronchial airways in mouse lungs. The images were of mice that had been exposed to cigarette smoke for 24 weeks (CIG) and of age matched control mice that had not been exposed to any cigarette smoke (AMC).The bronchial airways had to be differentiated from other lung parenchyma by comparing the types of cells surrounding the conducting airways and noting the presence of elastin on the walls of the veins and arteries which run parallel to the bronchi (Fig. 2B). ImageJ was also used to measure the size of the cells lining the bronchi and to measure airway thickness and area. This data was then analyzed to determine similarities and differences between AMC and CIG airways.

Figure 2:

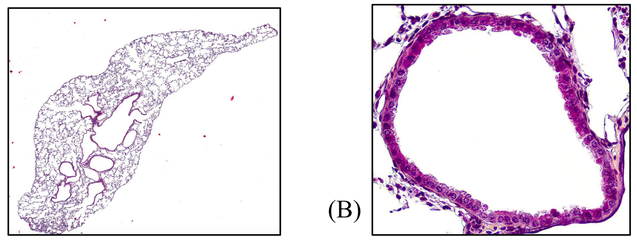
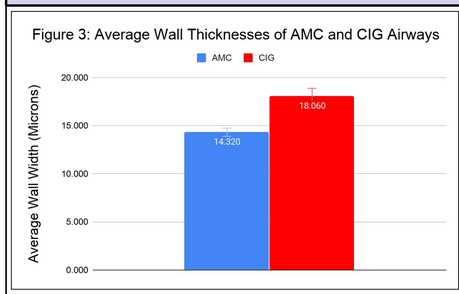
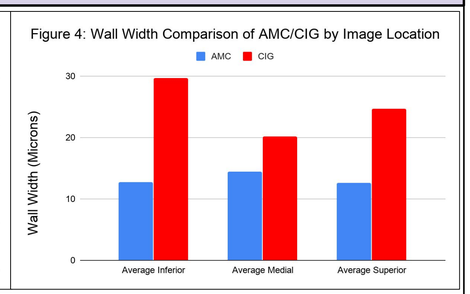
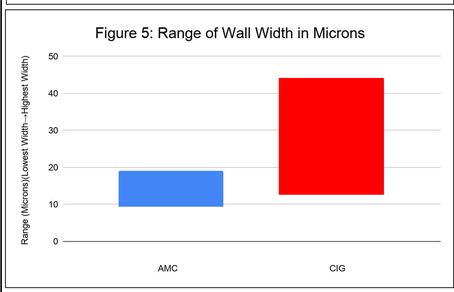


Figure 2: The entire superior lung section of AMC mouse 2 (A). A representative airway in the lung of AMC mouse 2 (Taken from a superior section of the lung) (B).

Results







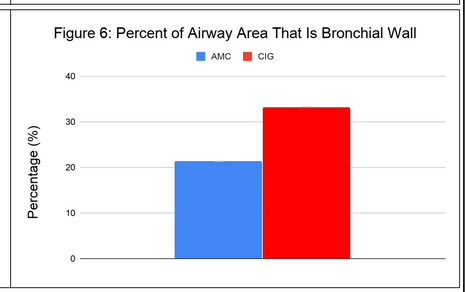


Figure 3: A statistical analysis comparing the average bronchial wall thicknesses of AMC and CIG mice. CIG airways are significantly thicker than AMC airways.
Figure 4: A more in depth comparison of the average bronchial wall thicknesses of AMC and CIG mice based on their location in the lung.
Figure 5: A comparison of the range of airway wall width in AMC and CIG mice. While the thinnest airway walls have similar widths between AMC and CIG, CIG mice have a greater range in their airway wall widths.
Figure 6: A comparison between the area of the bronchial wall and the area of the bronchi itself. AMC bronchi have higher ratio of airway area to wall area than that of the CIG bronchi.

Conclusion

In conclusion, the bronchial wall thickness, its overall area, and the percentage of the bronchi taken up by the bronchial wall increased after exposure to cigarette smoke in the CIG mice. These findings are statistically significant with $p<0.001$ for all findings. Knowing this, it is important to conduct more research in this area in order to determine whether or not the bronchial wall thickening (Fig. 7B) is reversible, potentially with cessation or through medical treatment. Furthermore, lung mucus production appears to have been affected by this exposure to cigarette smoke. While mice do not usually produce mucus in their lungs (Fig. 8A), mucus (stained for using the antibody MUC5AC) seems to be visible from the grey/brown color lining the bronchi in Figure 8B, which will be quantified next in this study.

Figure 7:

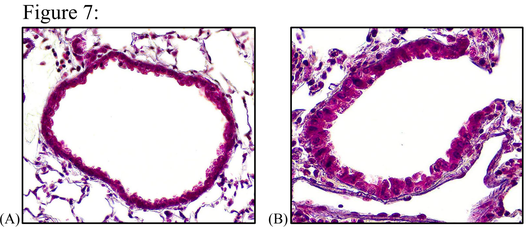


Figure 7: An example of a bronchi from an inferior section of an AMC lung (A). An example of a bronchi from an inferior histological image of a CIG lung (B).

Figure 8:

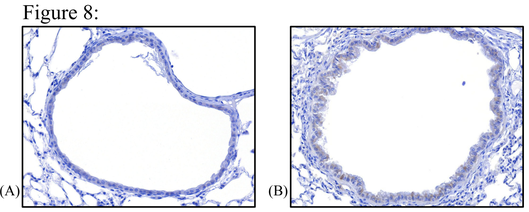


Figure 8: An immunohistochemical image of an AMC mouse bronchi (A). An immunohistochemical image of a CIG mouse with mucus production noted in brown/grey (B).

References

1) Charbonnier, J., Pompe, E., Moore, C., Humphries, S., van Ginneken, B., & Make, B. et al. (2019). Airway wall thickening on CT: Relation to smoking status and severity of COPD. *Respiratory Medicine*, *146*, 36-41. doi: 10.1016/j.rmed.2018.11.014
2) Chronic obstructive pulmonary disease (COPD). (2017). Retrieved 23 July 2020, from https://www.who.int/news-room/fact-sheets/detail/chronic-obstructive-pulmonary-disease-(copd)
3) Groneberg, D. A., & Chung, K. F. (2004). Models of chronic obstructive pulmonary disease. *Respiratory research*, *5*(1), 18. https://doi.org/10.1186/1465-9921-5-18
4) Hodge-Bell, K. C., Lee, K. M., Renne, R. A., Gideon, K. M., Harbo, S. J., & McKinney, W. J. (2007). Pulmonary inflammation in mice exposed to mainstream cigarette smoke. Inhalation toxicology, 19(4), 361–376. https://doi.org/10.1080/08958370601144076
5) Linnea Hedman. ERS Handbook of Respiratory Medicine. (2020). Retrieved 23 July 2020, from https://books.google.com/books?hl=en&lr=&id=7TKyDwAAQBAJ&oi=fnd&pg=PA257&dq=smoking+and+diseases&ots=KBtvQoHcJ0&sig=OEBIDs7WF7KLSZzBBgBS9_lxLEk#v=onepage&q=smoking%20and%20diseases&f=false
6) Popper H., Murer B. (2020) Smoking-Related Diseases. In: Moinfar F. (eds) Pulmonary Pathology. Essentials of Diagnostic Pathology. Springer, Cham. https://doi.org/10.1007/978-3-030-22664-0_22
7) Quick Facts on the Risks of E-cigarettes for Young People. (2020). Retrieved 23 July 2020, from https://www.cdc.gov/tobacco/basic_information/e-cigarettes/Quick-Facts-on-the-Risks-of-E-cigarettes-for-Kids-Teens-and-Young-Adults.html
8) Smoking and Tobacco: Data and Statistics. (2020). Retrieved 23 July 2020, from https://www.cdc.gov/tobacco/data_statistics/index.htm
9) The top 10 causes of death. (2018). Retrieved 23 July 2020, from https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death

Acknowledgements

Department of Bioengineering
Dr. Jessica Oakes - Assistant Professor
Jacqueline Matz - Ph.D. Candidate

Department of STEM
Claire Duggan - Director of Programs and Operations
Salima Amiji, Natasha Zaarour - YSP Coordinators