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Coláiste na Tríonóide, Baile Átha Cliath
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An Investigation into the Relationship between Type II Alveolar Epithelial Cells and Alveolar Macrophages in Patients Suffering from COPD.

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Introduction

Chronic obstructive pulmonary disease (COPD), is the third leading cause of death worldwide, contributing to 3.2 million deaths in 2017 [1]. It manifests as chronic bronchitis or emphysema and is a clinical syndrome characterized by breathing difficulties, a chronic cough and inflammation of the lungs. The most common causes of COPD are smoking and exposure to air pollution (harmful airborne chemicals), which translates to a larger number of expected deaths, due to rising levels of air pollution in the world. However, it is a field that is severely underfunded and under researched, thereby limiting our understanding of the disease and resulting in very few treatments being available.

So, what happens in the lungs to cause COPD? As air travels down the respiratory path, it enters the bronchi, bronchioles and finally the alveoli. This is where the exchange of oxygen and carbon dioxide occurs in our bodies. The destruction of these alveolar spaces is a key event in COPD. The alveoli are lined with different two types of epithelial cells, type I and type II. Type II cells are involved in gas exchange, regeneration of damaged type I cells and xenobiotic metabolism (breakdown of foreign substances). Type II cells also trigger the production of alveolar macrophages (AMs). AMs are white blood cells that control inflammation, and are the first cells to encounter pathogens, pollutants etc. The continuous exposure to cigarette smoke, air pollutants etc. leads to a drastic increase in the number of AM's which results in inflammation and contributes to damage of the alveolar endothelial cells, mucus hypersecretion, and limited airflow in the airways. Persistent exposure to irritants and ongoing inflammation is considered to be the aetiology of COPD.

In this research project, my major objective is to study the relationship between the type II alveolar cells and the AMs. By further understanding the interaction between these cells, we can potentially discover novel treatments for COPD.

I am also extremely passionate about evaluating the role of non-animal testing models in biological research. Currently, the majority of work on COPD requires the use of animal testing. A common technique used for COPD research is the inhalation method where this condition needs to be induced into mice, rabbits etc. This is done by exposing these animals to different pollutants such as cigarette smoke. Although this is one of the most accurate forms of testing, it is still extremely limited due to human- mouse differences.

Until recently modelling AM biology *in vitro* has been limited, given AMs are challenging to isolate and rapidly loose identity in standard culture. This process has also involved the use of excessive amounts of mice. The Cloonan lab has recently established an *in vitro* AM system using foetal derived alveolar macrophages (FLAMs) based on protocols from two recent studies (*Immunohorizons. 2022 Feb 22; 6(2) 156-16* and *Am J Respir Cell Mol Biol.*

2022 Jan;66(1):64-75). They have also been able to culture primary AMs directly from the murine lung (MexAMs). While these AMs are unfortunately still derived from animals, they both can be cultured for up to 6 months, and therefore can greatly reduce the number of animals required for testing.

During my six-week research project, I plan to work with my supervisor, Dr. Suzanne Cloonan who has applied for a grant funded by PETA. If awarded, she plans to purchase primary human type II cells and (AM's derived from human lungs cells), which can then be used instead of the mouse cells. My role would be to help conduct systemic literature search, analyse data on feasibility and hopefully also partake in the experimental studies.

Project Methodology and Timeline

No	Workpackage title	DURATION (WEEKS)	STARTING WEEK	Week					
				1	2	3	4	5	6
1	**Systematic Review**	**6**	**1**						
1.1	Literature survey	**3**	**1**						
1.2	Comparative analysis	**2**	**5**						
2	**Experimental Validation**	**6**	**1**						
2.1	Growth of FLAMs/ MexAMs/Ams	**2**	**1**						
2.2	Exposing AMs to cigaratte smoke	**1**	**2**						
2.3	Wash/ Co-culturing at air liquid interface*	**2**	**3**						
2.4	Analysis of Results*	**2**	**5**						

*An air liquid interface (see Figure 1 below) will mimic the alveolar microenvironment.

*To analyse the type II cells the methods used are:

- Measurement of inflammatory markers by real time (RT) quantitative PCR – RTqPCR or ELISA (enzyme-linked immunosorbent assay).
- Immunoblotting for type II cell identity markers including SPC and cell injury markers.

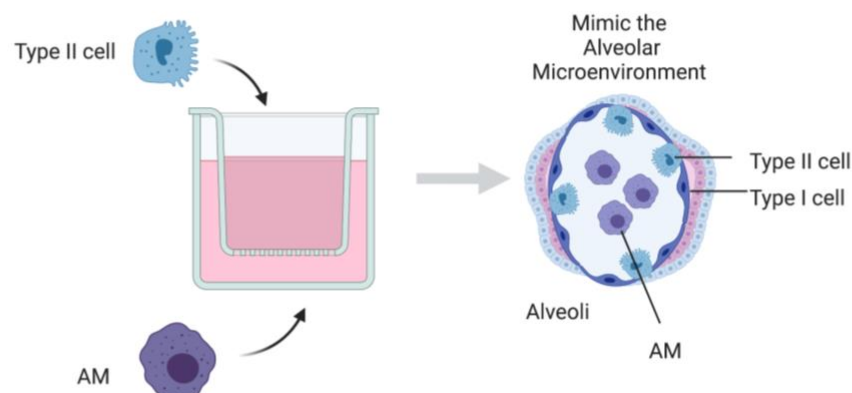

Figure 1 depicts the air liquid interface where AMs that have been exposed to cigarette smoke will be placed and co-cultured alongside type II cells.

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Intended Outcomes

As mentioned above, these experiments will investigate the effect of AMs on type II cells. If the relationship between these cells is understood, we will be closer to understanding how to reduce inflammation in lungs and a COPD patients' susceptibility to other infections.

The second intended outcome of my research will be to increase awareness of alternative to animal testing in biological research. By carrying out literature surveys and comparative analysis of the main testing methods, I hope to determine the most effective and cruelty free options of testing.