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Engineering the Pulmonary Blood-Gas Interface

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Introduction

Nanomaterials are prevalent in the environment, however their impact to human health is not fully understood. Current screening tools are either animal exposure studies or single phenotype in vitro assays. Animal studies are useful because they provide integrated tissue level toxicity metrics. However, they are expensive and cannot be scaled up for large scaled screening. In vitro assays are more cost effective and scalable, but cannot provide complex toxicity readouts.

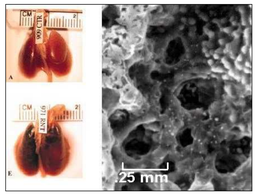

Fig. 1 Effects of carbon nanotubes

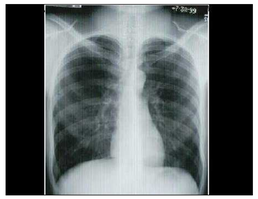

Fig. 2 X-ray of human lungs after being exposed to nanomaterials

The goal of this study is to engineer a microfabricated device that mimics the gas-blood interface of the lung, which is shown in Fig. 3. This interface is essential to gas exchange to the blood and is susceptible to damage from nanomaterial exposure. A schematic of the proposed device is shown in Fig. 4.

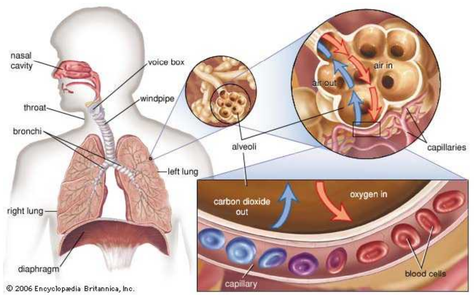

Fig. 3 The pulmonary blood-gas interface

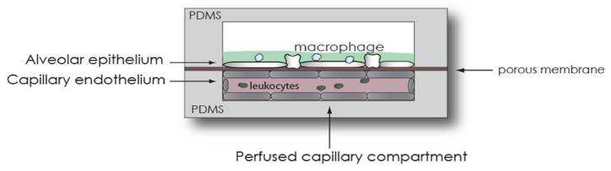

Fig. 4 Cross-section schematic of proposed device

Membrane Creation

In the lung, the alveolar epithelial and capillary endothelial cells are separated by a submicron thick membrane, which allows for diffusion of nutrients, but not the cells. In order to mimic this function, the membrane has to be thin, but strong enough to support cell growth on both sides. This membrane is created through the supramolecular self-assembly of hyaluronic acid (HA) and a peptide amphiphile (PA), that was previously described in Capito et.al (2008). HA is a negatively charged molecule that contains repeats of *N*-acetylglucosamine and glucuronic acid and is shown in Fig. 5. A peptide amphiphile is a smaller molecule with a peptide head that forms beta sheets and a hydrophobic tail and is shown in Fig. 6.

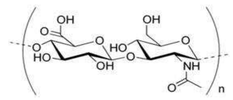

Fig. 5 Basic structure of HA (Capito et. al)

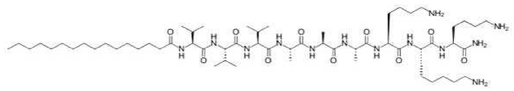

Fig. 6 Structure of PA (Capito et. al)

For this experiment, the peptide had the sequence $V_3A_3K_3$ with a 16 carbon tail attached to the last valine. The PA was synthesized using solid phase peptide synthesis, which is shown in Fig. 7.

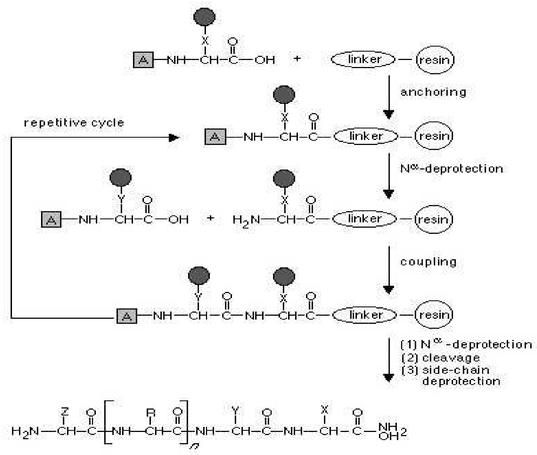

Fig. 7 Basic solid phase peptide synthesis (Fields, 2002)

The initial membrane is formed by PA nanotubes at the interface, then the HA diffuses into the PA solution. The PA is attracted to the HA and the two molecules form nanofibers. This process can be seen in Fig. 8.

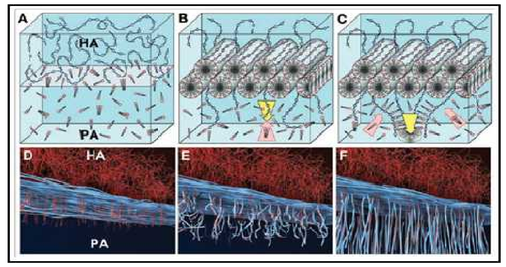

Fig. 8 Model of HA/PA self-assembly (Capito et. al)

We created a suspended membrane to incorporate into the device. A complete membrane can be seen in Fig. 9.

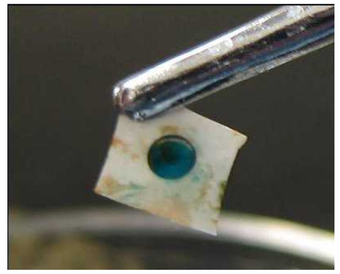

Fig. 9 Self-assembled membrane shown in blue. White outer portion used to transfer membrane to device.

Device Fabrication

In order to determine if cells can be grown on devices, A549 lung cells were seeded onto the membrane via the access port. The device is shown in Fig. 10. The two liquids are kept separate by the porous membrane.

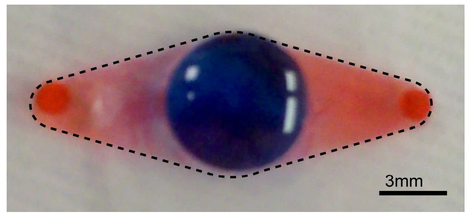

Fig. 10 Device with the bottom channel filled with red food color and top channel filled with blue food color

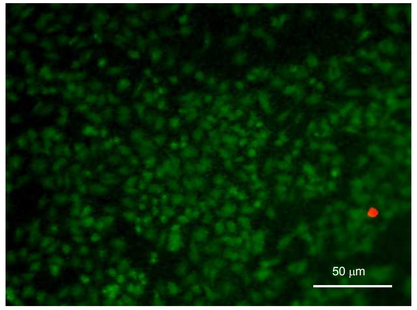

Fig. 11 Live/dead stain of A549 cells at 10x (live is green and red is dead)

The devices were first incubated with Geltrex to assist in cell adherence. The cells were then seeded onto the membrane at 15,000 cells/well and allowed to incubate. A live/dead stain was performed to ensure viability of cells.

Ongoing Research

Currently, immortalized type 2 epithelial cells are being characterized through Western blots and q-PCR. Primary type 1 and 2 cells will be characterized through the same processes. Once the device is ready and the cells are fully characterized, they will be exposed to silicon dioxide and zinc oxide to determine the toxic effects of zinc oxide.

References

(1). Capito, R. M., H. S. Azevedo, Y. S. Velichko, A. Mata, and S. I. Stupp. "Self-Assembly of Large and Small Molecules into Hierarchically Ordered Sacs and Membranes." *Science* 319.5871 (2008): 1812-816.
Photo credits:
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Acknowledgements

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