ENDOTHELIALIZED MICROFLUIDIC DEVICES FOR STUDYING PULMONARY VASO-OCCLUSION

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INTRODUCTION
Sickle Cell Disease (SCD) is an autosomal genetic disease that affects 1 in 500 African Americans and millions across Sub-Saharan Africa. Hemoglobin is the protein within the red blood cells (RBCs), which transports oxygen through the blood to distant tissues and organs. SCD arises due to a single nucleotide mutation in the β-globin gene and leads to sickling of red blood cells under deoxygenated conditions. Following deoxygenation, the mutated hemoglobin (HbS) undergoes polymerization, and forms long fibers that distort the cell membrane causing the RBC to sickle. The process of sickling causes RBCs to become rigid and more adhesive than the healthy red blood cells due to enhanced expression of adhesion molecules not expressed on mature healthy RBCs. The enhanced rigidity and adhesiveness of sRBCs can increase their transit time through small blood vessels and may allow them to adhere to neutrophils (most abundant leukocyte in human blood) and endothelial cells (cells lining the inner wall of a blood vessel), eventually leading to vaso-occlusion (blockage of blood vessels). Vaso-occlusion is believed to be responsible for the acute pain crisis and the acute chest syndrome (ACS), the two main pathologies responsible for morbidity and mortality among SCD patients. ACS is a type of lung injury, which happens only in SCD patients. Animal studies have revealed a role for neutrophils and sRBCs aggregation in vaso-occlusion in different organs of the body, but the evidence to support the role of vaso-occlusion in ACS does not exist. Preliminary data using SCD mouse model from our lab reveal a role for neutrophil-sRBC micro aggregates in vaso-occlusion in the lung. However, it is not known whether such aggregation can happen in human blood vessels. I am proposing to establish an in vitro assay that can be used to test the relevance of neutrophil-sRBC micro-aggregation leading to vaso-occlusion in lung capillaries of humans. The lung microcirculation consists of small capillaries (5-10 μm inner diameter), which are smaller than both RBCs and neutrophils. As a result, the transit of these cells through the lung requires them to deform and flow like a ‘slug’ within the capillaries.

OBJECTIVE
Human lung microvascular endothelial cells (HMVEC-L) will be cultured in microfluidic channels that mimic the capillary structure found in human lungs. The endothelialized microfluidic channels will be perfused with fresh anti-coagulated blood from healthy or SCD patients to create a physiologically relevant environment to study vaso-occlusion.

HYPOTHESIS/SUCCESS CRITERIA
It is hypothesized that microfluidic channels cultured with human lung endothelial cells (HMVEC-L) will serve as a clinically relevant model to study pulmonary vaso-occlusion in whole blood from Sickle Cell Disease patients. In addition, it will be tested whether 7-10 μm polydimethylsiloxane (PDMS) channels can be cultured with HMVEC-L and used as an in vitro model for pulmonary vaso-occlusion.

METHOD
The human lung endothelial cell line HMVEC-L will be cultured to confluence within a PDMS microfluidic device. Microfluidic chips with inter-connected microchannels that mimic the structure of the lung capillary network will be used as seen in Figure 1. The chips will be created using soft-lithography and calibrated to achieve desired flow as a function of hydrostatic pressure difference between the inlet and outlet of the device. The micro-channels will be coated with fibronectin (100 μg/ml) using a syringe pump. The endothelial cells will be maintained using standard culture methods in a flask in an incubator (37 °C, 5% CO₂). Once the cells are confluent, they will be resuspended in media at the concentration of 2x10⁷ cells/mL. The cell suspension will be transferred to a syringe and then placed in a syringe pump to begin perfusion through the device at 10μL/min. The cells must be allowed to adhere to the device for at least 4 hours at 37°C before changing the media. Once the cells are properly seeded within the device it is important to culture HMVEC-L under flow conditions at 1μL/min to maintain the most physiological environment.

Figure 1. Auto-perfused microfluidic device mimics pulmonary capillary system.
Once the micro-channels are cultured to confluence with HMVEC-L, they will provide a clinically relevant model for the lung capillary structure. The endothelialized lung-like microchannels will be perfused with SCD patient whole blood, which is heparinized to prevent coagulation. The cell to cell interactions between the endothelial layer within the device, sRBCs, and neutrophils will be observed using a high-speed multi-channel fluorescence microscope.

**RESULTS**

It was found that HMVEC-L reached confluence within the microfluidic devices between day 3 and 4 after cell seeding. Confluence was measured using a phase contrast microscope to estimate overall cell coverage. Attachment of the cells to the walls of the channels was evident 24 hours after seeding and cells continued to grow to confluence. Cells survived best within the devices when media was changed every 24 hours. The cell line HMVEC-L was no longer viable after 6 days of culture and all cells within the channels appeared dead.

**DISCUSSION**

The results of this pilot study are encouraging. Further study is required to perform statistical analysis to determine whether the hypothesis can be supported.

Endothelial cells may not grow in microchannels smaller than 7 μm. To circumvent this hurdle, HMVEC-L was grown in microchannels of 10-12 μm inner diameter. Once the endothelial cells are confluent, the actual diameter of the microchannel will reduce to 8 μm, which is still within the range of size for lung capillaries. This was completed successfully and now further study can be continued with channels that are 7-10 μm inner diameter.

In order to support the initial hypothesis and aims a confocal z-scan needs to be performed to confirm 3D confluence within the microchannels of the device. Initial confluence was confirmed using a standard light microscope at different focal planes. Using a confocal z-stack will provide conclusive support to the confluence of the device.

Parameters regarding the initial concentration of cell seeding also need to be optimized to reliably reach 3 days to 100% cell confluence.

This study was limited by a few factors. Despite attempting to maintain accurate human physiology, the system is in vitro and will not fully mimic a human study. Patient blood may coagulate within the device and can cause the flow through the device to stop. To avoid coagulation, blood from SCD patients will be collected in a 10 ml syringe containing 400 μl of clinical grade heparin. The blood will be allowed to gently flow in the syringe by controlling the movement of plunger.

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**REFERENCES**

