Isolation of Low Frequency Cells in Bronchoalveolar Lavage (BAL)

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Abstract

The fourth leading cause of death today in the United States is lung disease which is quite often treated by lung transplantation. Lung transplant recipients seem to carry a greater risk of developing obliterative bronchiolitis (OB) which has been linked to chronic rejection of the transplanted lungs. Some recent studies have produced data that suggests the number of fibroblast and epithelial cells present in bronchoalveolar lavage (BAL) correlates to the severity of OB. Establishing a biomarker that can be used in the diagnosis of OB is important, because no clinical test exists that can be used to directly diagnose the disease. In these studies we show the populations of low frequency cells (fibroblast and epithelial) within different BAL samples. Cells are counted and sorted by type via a panning method and work has been done to prove their phenotype with immunostaining. This is an important step as no other research has documented the relative frequencies of cell populations within BAL samples, and provides a foundation to develop a clinical assay for detection of those subpopulations.

Separation Methods Investigated

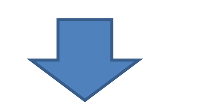
Immunomagnetic Separation
•Yielded inconsistent results
•Cell populations at the edge of the technique's ability to separate
Panning Method
•Tool used to determine cell populations within the BAL
•This information from BAL samples has yet to be characterized

Hypothesis

Epithelial and fibroblast cells make up roughly 1% of the BAL cell population and can be isolated by use of existing techniques.

Panning method of separation

1) The BAL sample is first placed into an IgG coated 6 well plate and allowed to incubate for 1.5 hours.



2) The supernatant is removed from the IgG plate, centrifuged and reseeded onto a tissue culture treated petri dish for another 1.5 hours.

3) The supernatant is once again removed from the second step, and transferred to a fibronectin coated petri dish

This method allows for macrophages to be isolated in step 1, fibroblasts in step 2, and epithelials in step 3.

Acknowledgements

Thank you to Dr. Hubel for giving me the opportunity to do this research and for the help of Sanket Unhale with this project.

References

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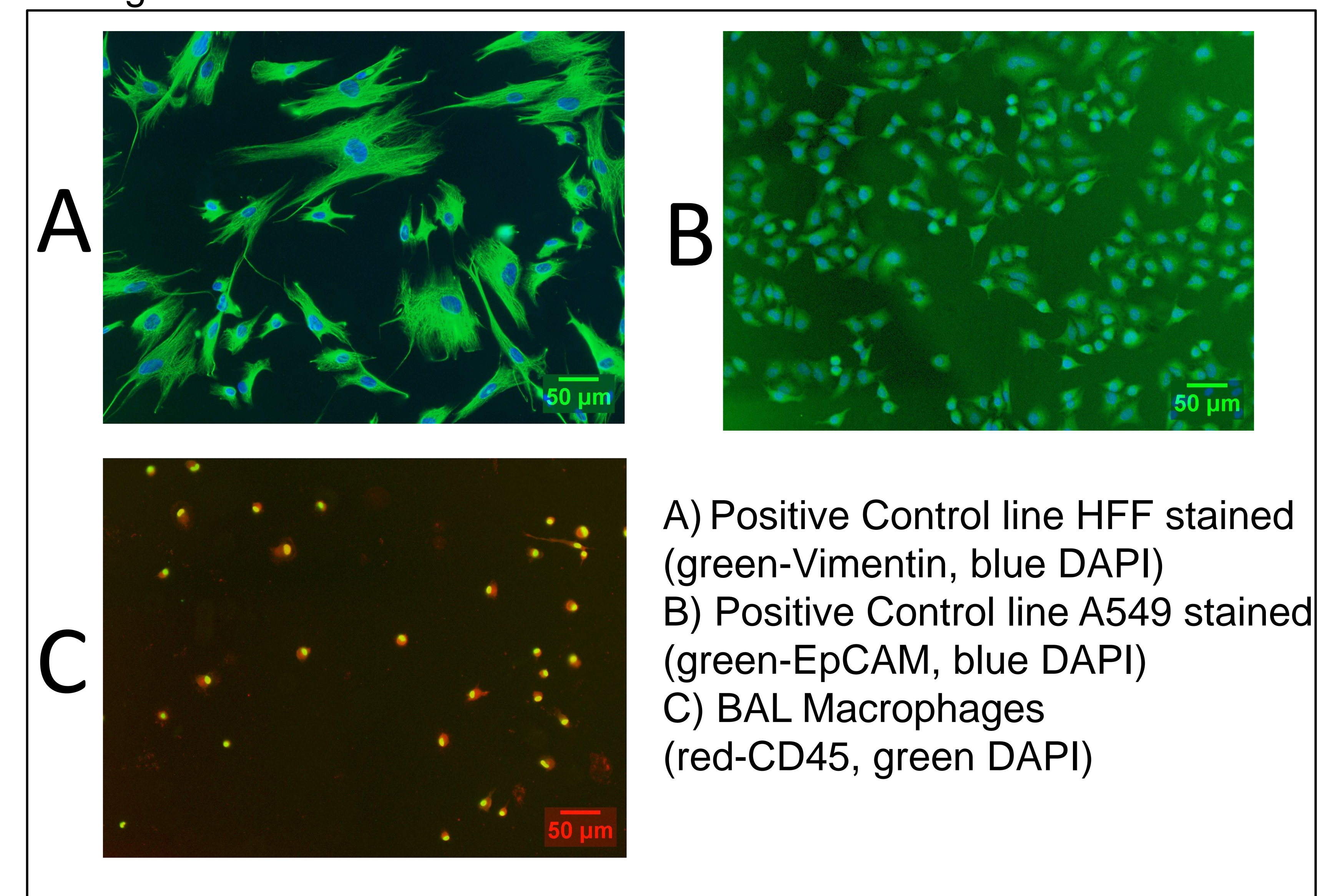
Results

Table 1

(n=5)	Percentage of Cells Present	Average Cell Count
IgG Coated (macrophages)	98.49%	1,056,686 +/- 569,097
Tissue Treated (Fibroblasts)	0.85%	11,228 +/- 15,180
Fibronectin Coated (Epithelial)	0.67%	5,928 +/- 2,839

Figure 1



A) Positive Control line HFF stained (green-Vimentin, blue DAPI)
B) Positive Control line A549 stained (green-EpCAM, blue DAPI)
C) BAL Macrophages (red-CD45, green DAPI)

Future Direction

The groundwork has been laid in determining the frequency of epitheal and fibroblast cells within BAL samples. Positive controls cell lines have been obtained and tested with the desired antibodies to prove the phenotype; all that remains is performing immunostaining on a BAL sample. Following this, flow cytometry will be used to further confirm these results. Ideally, a micro-fluidic device will be designed to incorporate the panning procedure into a simple clinical test that can be used to determine the severity of OB.