Title

3D printing aids simultaneous isolation of proximal and distal lung epithelial progenitors from individual mice

Abstract

The lung epithelium is rich in cell type diversity including several progenitors. These distinct regional progenitors have traditionally been identified using lineage tracing or single cell RNAseq. Isolation and use of such cells are hampered by the lack of specific markers that distinguish them in different airway compartments. Thus, a robust method is needed to reliably isolate progenitors from their respective location in the airway. Further, isolation of cells from the same mouse allows for direct analysis of regional disease specific effects and reduction in overall animal numbers. Here, we developed a 3D-printed lobe divider (3DLD) to simultaneously isolate proximal and distal epithelial cells from the same lung. We evaluated isolated cells by their capacity to differentiate in standard *in vitro* assays. Proximal and distal cells isolated with 3DLD gave rise to differentiated airway and alveolar epithelia respectively as evident by air-liquid-interface cultures and organoid assays with colony forming efficiency (CFE) matching literature. 3DLD method yielded distal organoids with increased CFE and smaller organoid diameters with median of 107µm compared to 171µm from those isolated without 3DLD. We used RNAseq in combination with computational deconvolution to evaluate differences in cell type representation among these cultures. We found that separation of proximal and distal compartments prior cell isolation altered the initial cell populations, likely due to the presence of proximal airway progenitors in single cell suspensions of distal epithelial cells. 3DLD is an inexpensive and reproducible method for isolating proximal and distal progenitors from individual mice.