A biomimetic multicellular model of the airways using primary human cells

1. Long-term culture flow apparatus
To achieve passively driven fluidic systems for long-term perfusion that can be readily multiplexed for parallelization of the cultures, we adopted constant-flow syringes to keep a constant pressure head at the inlet and, importantly, included a wick in the outlet syringe. We found that the wick needed to be kept hydrated and in constant position relative to the outlet medium level to enable flow. Different combinations of tubing material (polyurethane, silicone and polyethylene) and constrictions (PEEK and stainless steel microbore tubing) were tested in pilot experiments. Polyurethane tubing with a stainless steel constriction was found to be the least prone to bubble formation. Bubble formation causing flow restriction was observed on average every few days. If addressed by daily inspection, bubbles were not detrimental to the cultures (Figure S1B). The reservoirs were typically filled to allow at least 3 days between refills. The custom system using “constant flow” nested syringes at the inlet and an outlet at fixed height difference $\Delta H$ is shown in figure S1(A). The gravity flow provided under the conditions of a $\Delta H$=10mm and with a 200 mm long 150 $\mu$m diameter constriction (1/32" OD stainless steel, U-1122 Idex) was estimated by the liquid volume decrease in the inner nested inlet syringe (Fig. S1).

Fig. S1 (A) Photograph of a gravity driven flow system connected to a three layer device, with perfusion flow for two compartments, each with one inlet and outlet syringe. (B) Example of
flow rate for two compartments measured over a culture period of 23 days. The flow was estimated by the decrease in medium volume in the nested inlet syringe reservoir, the errorbars on the first points in the graph indicate the precision of the measurement. Arrows indicate medium refills.

2. **Histology cross-section of AE cultures grown on PTFE and PET cell inserts.**

Histological section of AE cells grown on PET (Snapwell) and PTFE (Millicell) membranes demonstrate that a thicker and more stratified cell layer is obtained on PTFE.

![Graph showing cell layer thickness](image)

**Fig. S2** (A) Cell layer thickness obtained from cultures of the same age on both membrane types and from nine separate donors. (B) and (C) Optical transmission microscope images of histological sections on PTFE and PET membranes.
3. Bonding strength measurement
To measure the maximum pressure the microfluidic devices were able to resist without membrane delamination or loss of pressure, air was introduced into the inlet tubing of the bottom channel at a flow rate of 2 mL/min using a syringe pump (Harvard Apparatus). The outlet tubing was connected to an analog pressure sensor with a maximum pressure reading of 100 psi (689 kPa). Devices with channels sandwiching a membrane had the inlet and outlet of the upper channel blocked by completing the channel loop to allow pressure to build within the system. The maximum pressure was recorded as the pressure immediately prior to PDMS delamination. Tests were only considered valid for trials where either the bonded PDMS or membrane delaminated: experiments where leakage occurred at the fluidic interface connections were disregarded. All reported results in Table S1 are an average (± st. dev.) of at least three measurements.

Table S1. Burst pressure (in kPa) measured for dry PDMS devices with different membranes and bonding treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Membrane type</th>
<th>PTFE</th>
<th>PC</th>
<th>No Membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td>APTMS + compression</td>
<td></td>
<td>91.2±25.5</td>
<td>-</td>
<td>296.5±3.4</td>
</tr>
<tr>
<td>APTMS + compression + thermal</td>
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<td>115.8±17.9</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Plasma only</td>
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<td>-</td>
<td>317.2±13.8</td>
<td>298.5±32.4</td>
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<tr>
<td>Aging (21 days in incubator)</td>
<td></td>
<td>73.8±35.9</td>
<td>227.5±120.7</td>
<td>268±88.9</td>
</tr>
</tbody>
</table>

4. Microfluidic cell cultures of individual cell types: fibroblasts and microvascular endothelial cells
Figure S3 shows fibroblast and microvascular endothelial (MvE) cells cultured on a membrane in two-layer single-membrane microfluidic devices, respectively PTFE and PET membranes.
Fig. S3 (A) Fb on PTFE membrane two-layer microfluidic device in ALI 1 medium at day 5, green phalloidin staining; (B) MvE on PET membrane two-layer microfluidic device in EGM medium at day 7 (green phalloidin and blue Hoechst nuclear staining). Scale bar 50 μm.

5. Effect of medium choice on MvE cells

Figure S4 illustrates the increased MvE cell diameter in all media compared to their optimized EGM media. Live-dead staining (Figure S4 A-C) shows the poor viability in ALI 1 as compared to EGM and 50:50 EGM: ALI 1 cultures of the same age.

Fig. S4 Effect of medium on MvE cells cultured in collagen coated plastic wells plates at day 7. Live dead staining of MvE cells in (A) EGM, (B) 50:50 EGM:ALI 1 (C) ALI 1. Scale bar 200μm. Phalloidin staining of MvE cells cultured in (D) EGM and (E) ALI 2. Scale bar 100 μm. (F) Cell number over area measured at day 7 in cultures grown in different media as indicated.