

Breathing Life into Innovation: Developing an Advanced COPD Lung Model for Therapeutic Discovery

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Methods

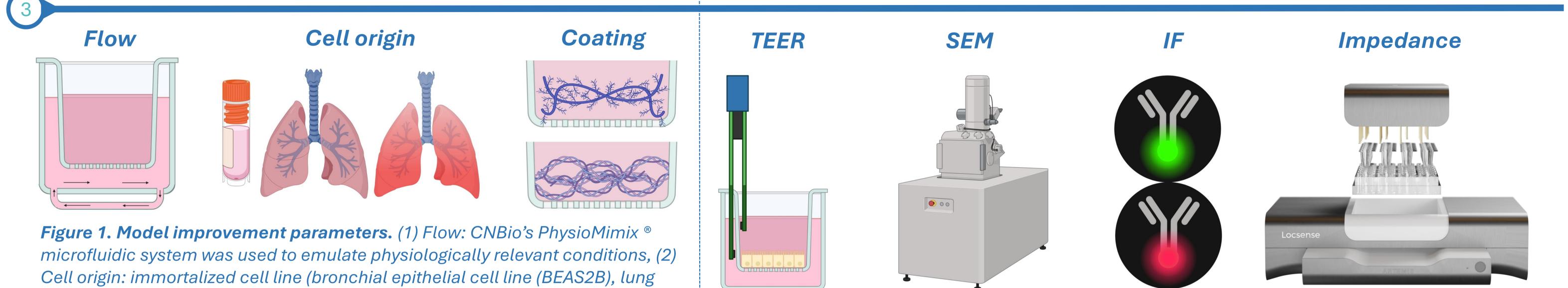


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- COPD is a major global cause of mortality, but current treatments only provide symptomatic relief.
- Limited progress in developing new therapies is attributed to the absence of reliable lung models for testing.

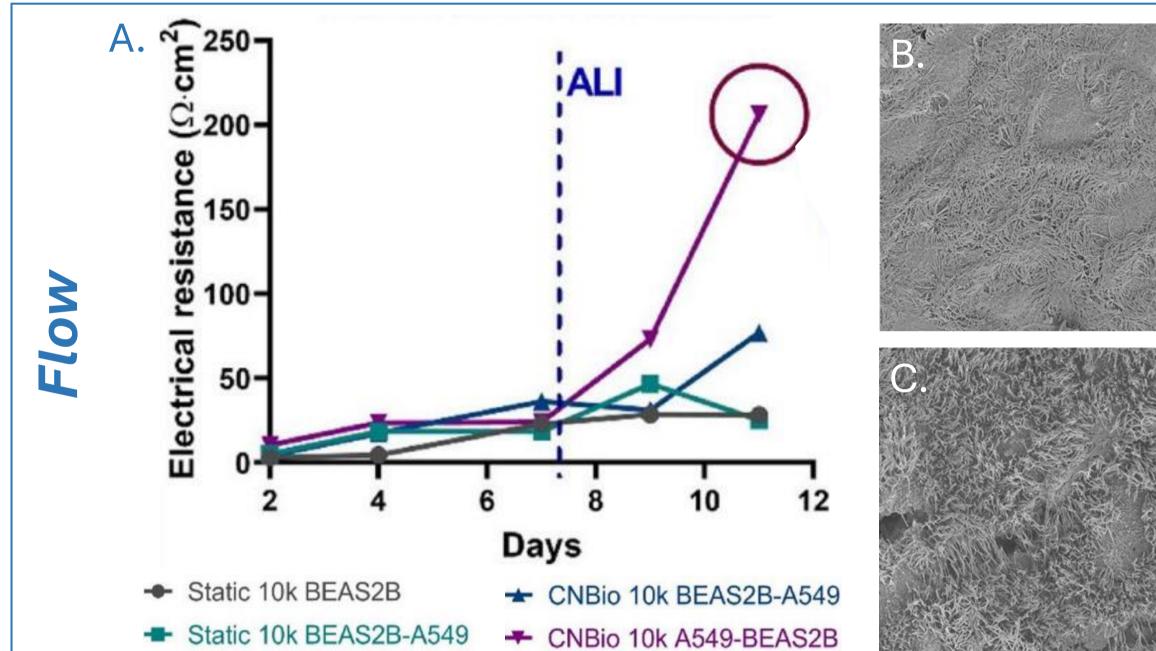
We formulated a healthy and COPD *in vitro* lung mimic that incorporates (1) microcirculation (2) extracellular matrix (ECM) and, (3) a diseased **COPD** primary cell line. To assess our model, we created an extensive quality control (QC) for barrier integrity and physical characteristics.



cancer cell line (A549)), were compared to healthy primary cells (normal human bronchial epithelial cells (NHBEs) normal human lung fibroblasts (NHLFs)), and diseased primary cells (diseased human bronchial epithelial cells (DHBEs)), and (3) Membrane coating: fibronectin and collagen-I were compared to replicate biochemical aspects

Figure 2. Quality control. (1) Transepithelial electrical resistance (TEER) to assess barrier function, (2) scanning electron microscopy (SEM) to analyze the cilia of the cells, (3) Immunofluorescence (IF) to analyze ZO-1 tight junction expression, and (4) full spectrum impedance spectroscopy to analyze cellular development.

Results



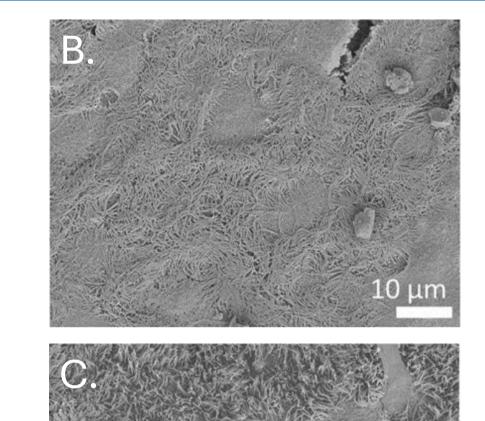
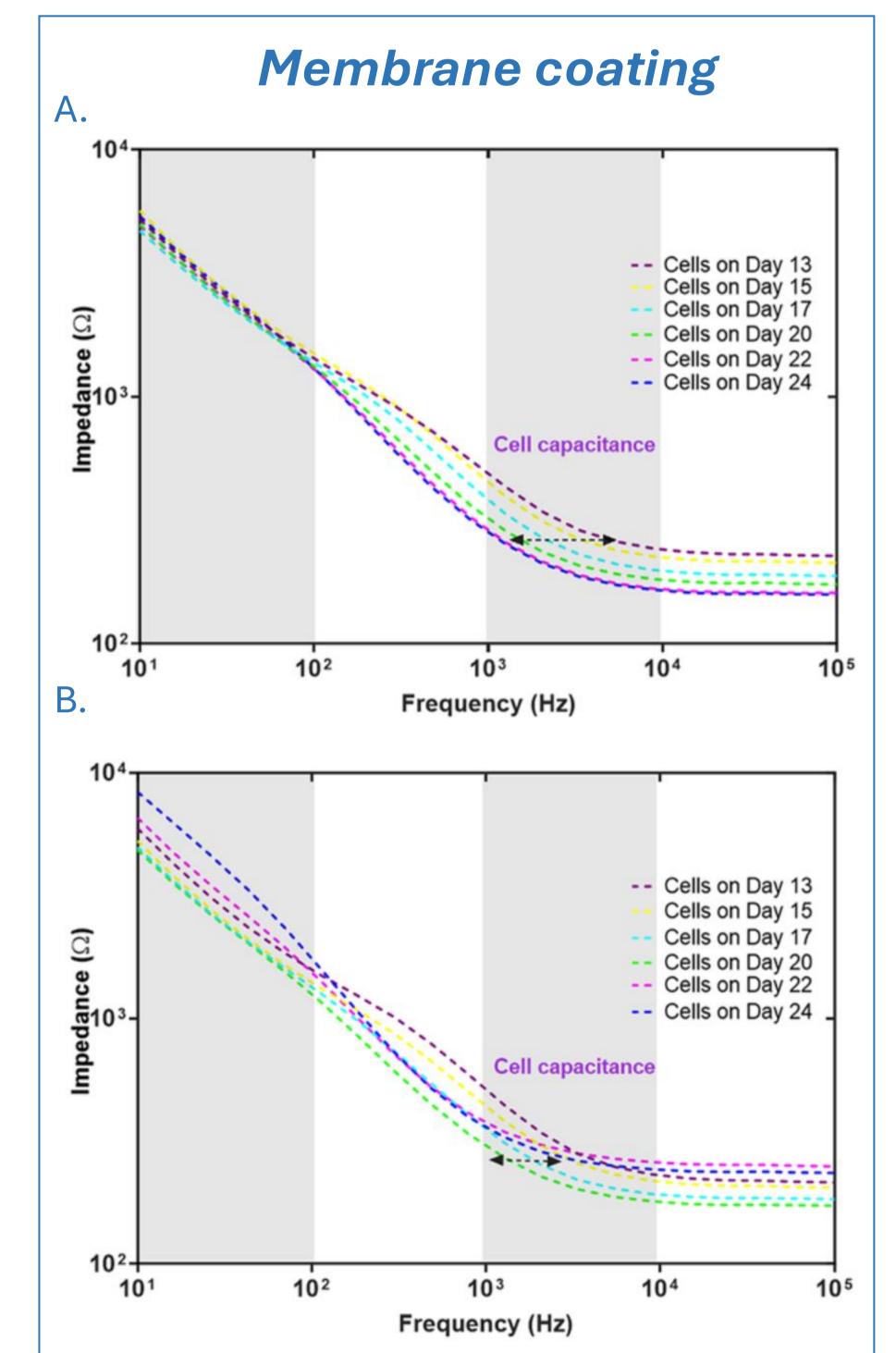


Figure 3. Dynamic flow increases TEER and induces lung-like hairy microvilli. A. Chop-stick TEER measurement of BEAS2B-A549 co-cultures. CNBio = $1 \mu L/s$ flow, B. Static NHBE-NHLF culture, C. CNBio flow NHBE-NHLF culture. The dynamic models (CNBio models) had higher TEER compared to static culture, indicating improved the cellular barrier





showed more cilia in the dynamic culture than in the static culture, indicating fully differentiated airway epithelial cells in dynamic cultures.

50 µm

50 µm

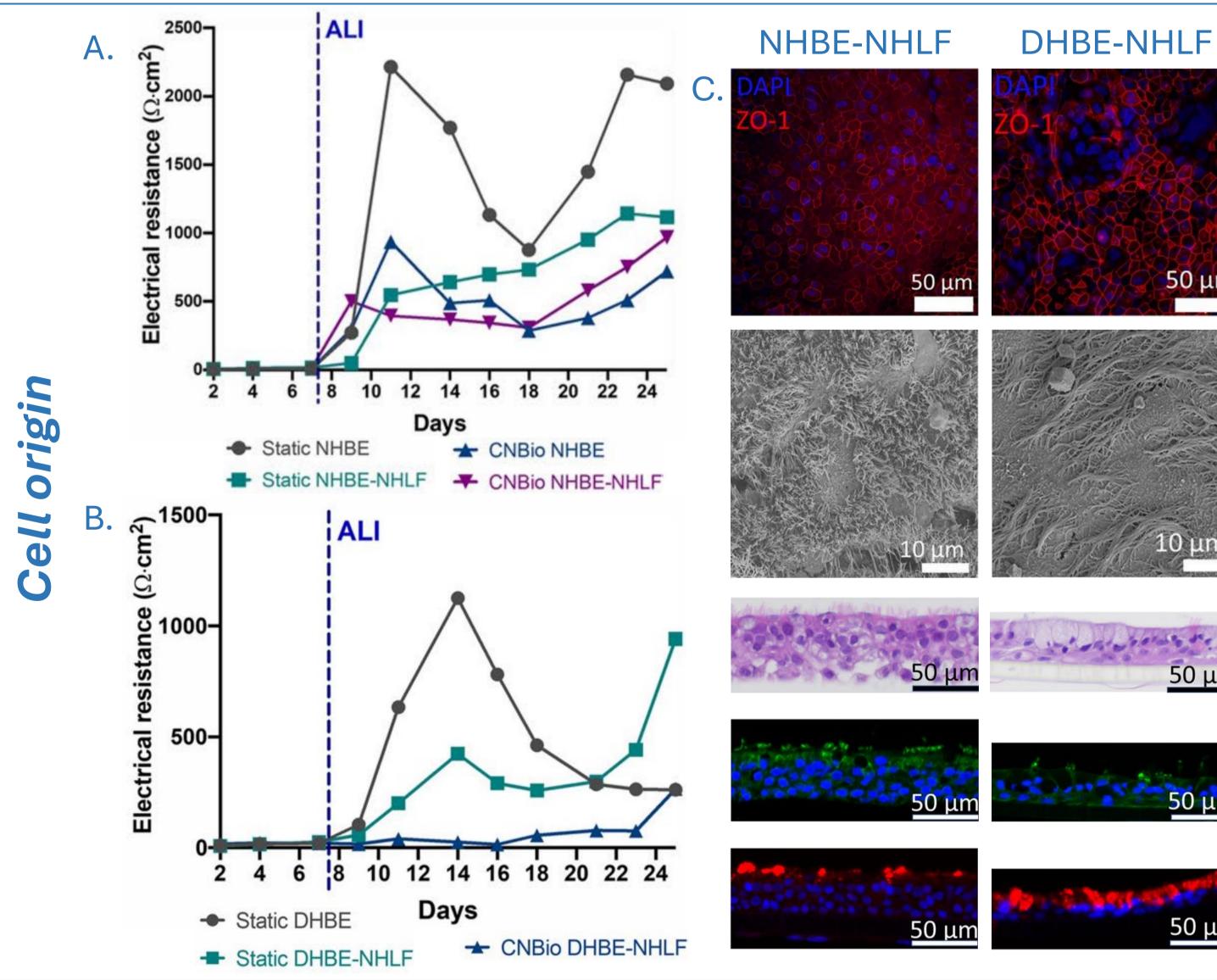


Figure 4. Incorporation of diseased cells leads to heterogeneous barrier A. Chop-stick TEER measurement of healthy culture, B. Chop-stick TEER measurement diseased culture, C. IF staining ZO-1, SEM, H&E staining, IF staining acetylated a-tubulin (cilia green), MUC5B (goblet cells – red). CNBio co-culture showed in vivo TEER results (~700 Ω *cm²). The ZO-1 tight junction marker and cilia homogeneously expressed in the healthy model and nonhomogeneous in diseased model. Moreover, in the diseased model, the ciliated epithelial cell are replaced by

Figure 5. Full spectrum impedance analysis A. 3 mg/mL collagen type I coating, day 13-24, B. B. 2.5 µg/mL fibronectin coating, day 13-24. More complex cellular structures leads to increased capacitance and thereby a drop in impedance. Concluding from the results, fibronectin, had a delayed drop, thereby indicating, reduced cell differentiation, compared to collagen which better induced cell differentiation.

mucus producing goblet cells, indicating an in vivo like diseased model.

Conclusions

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- Dynamic flow improved the barrier function and cell differentiation
- Collagen-I coated Transwells improved cell differentiation
- Replacing immortalized cell lines with primary cell improved the barrier and differentiation
- Using diseased human bronchial/tracheal epithelial cells COPD was effective in recapitulating the features observed in COPD patients



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