

Towards a Bronchus-on-a-Chip Device with Integrated Sensing for Monitoring of the Cell Culture

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Summary:

This work presents a proof-of-concept Organ-on-a-Chip platform for culturing epithelial bronchial cells and modelling bacterial adhesion in the human airway epithelium under physiologically conditions. The project focuses on device production and integration of an interdigitated electrode sensor, fabricated through lift-off process. The system aims to overcome the limitations of conventional static in vitro models by incorporating continuous flow. Future developments will assess anti-adhesive therapies.

Keywords: Microfluidics, Cyclic Olefin Copolymer, Impedance sensing, Interdigitated electrodes, 16HBE14o- cell line

Background, Motivation and Objective

Respiratory infections are a major global health concern, particularly in patients with chronic lung diseases such as cystic fibrosis, where impaired mucociliary clearance promotes persistent bacterial adhesion and colonization. Among opportunistic pathogens, certain species pose a severe threat due to multidrug resistance and association with life-threatening complications [1]. However, these processes remain poorly understood, mainly due to limitations of conventional models, which fail to reproduce dynamic airway conditions [2].

In this work, we propose a microfluidic Organ-on-a-Chip (OoC) device integrating impedance-based sensors for real-time, label-free monitoring of epithelial barrier formation and bacterial adhesion under physiological flow and oxygen gradients. To achieve this, two designs were developed: a hybrid system exploiting differential gas permeability of the two materials, and a device relying on controlled perfusion of media with different oxygen levels, a key novelty.

Finally, the platform is intended as a versatile tool to investigate bacterial adhesion under flow in physiologically relevant disease models, enabling the study of host–pathogen interactions and supporting the therapy development for infectious and epithelial barrier-related diseases.

Description of the New Method or System

The coexistence of normoxic and hypoxic conditions within a single device is replicated in novel microfluidic platforms, developed for adherent 16HBE14o- epithelial cells, featuring an 800 µL culture chamber to support monolayer formation and ensure compatibility with standard epithelial culture conditions.

The hybrid device (Fig. 1A) is composed of 2.5 mm PDMS (polydimethylsiloxane) and COC (cyclic olefin copolymer) layers and a PET (polyethylene terephthalate) membrane (0.4 µm pore size) is placed in between, supported by micropillars of the PDMS part.

In the fully COC-based device (Fig. 1B), a flat COC surface (0.1 mm foil or 2.5 mm plate) is associated with a machined plate, which includes microchannels for the inflow and outflow of two different culture media.

COC structures are fabricated by CNC (Computer Numerical Control) milling, while the PDMS components are produced via soft lithography, involving hard mask fabrication followed by SU-8 mold processing. Bonding between the two components uses a 143 µm-thick PSA (pressure-sensitive adhesive) layer.

Device fabrication is followed by design and fabrication of gold interdigitated electrodes (IDE) by photolithography (pre-development time: 40s, exposure energy: 75 mJ·cm⁻²), development,

metal deposition (10 nm Cr and 100 nm Au), and lift-off. Sensors were designed with 5 μm and 50 μm spacing with linear or circular fingers. The epithelial bronchial cells used, derived from the 16HBE14o- cell line, are seeded in the devices with a density of 25000 cells/cm². Biological validation include coated (LHC basal medium, BSA, collagen, human fibronectin) and uncoated conditions, with optical cell tracking.

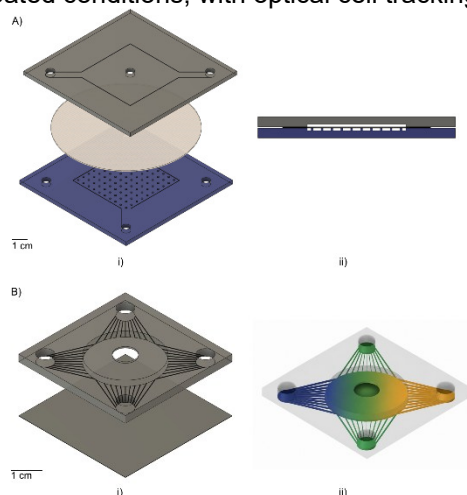


Fig. 1: Microfluidic devices

A) Hybrid PDMS-COC device:

- i) Schematic representation of different device layers,
- ii) Assembled device cross view

B) Full-COC device:

- i) Schematic representation of different device layers,
- ii) Illustration of O₂ gradient (normoxia (blue), hypoxia (yellow))

Results

Fabrication results indicate that CNC milling is the most reliable method for rigid thermoplastics devices, providing high-precision rapid prototyping, and that PDMS casting allows reproducibility using a master mold. PSA provides reversible sealing and fast assembly, and its replacement does not affect components, allowing device reuse and improving cost-effectiveness for iterative workflows.

Biological tests confirm adhesion and growth of 16HBE14o- cells, with better performance on COC flat surfaces, validating material compatibility for airway epithelial models (Fig. 2). Surface coating improves adhesion but is not essential for cell viability, as cells exhibit the expected phenotype. Physiologically relevant oxygen gradients mimicking in vivo bronchial conditions are under evaluation.

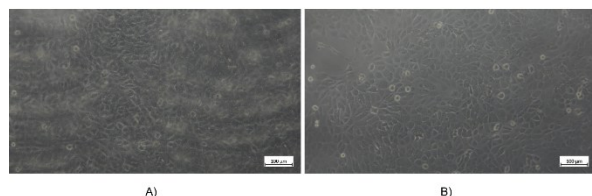


Fig 2. Micrograph (Bright-field, 10x magnification) of cells adhered at day 5 to non-coated (A) PET membrane (PDMS-COC device) and (B) COC foil (COC device)

A key outcome is the integration of IDE sensors, enabling quantitative and non-invasive analysis of cell behaviour and tracking responses to stimuli in future experiments.

The fabricated IDE sensors, with electrode spacings of 50 μm and 5 μm (Fig. 3, Fig. 4), exhibit uniform and well-adhered metallic layers, confirming fabrication protocol suitability. Future results are expected to identify which design performs best in terms of sensitivity and stability for real-time impedance monitoring of epithelial barrier formation.

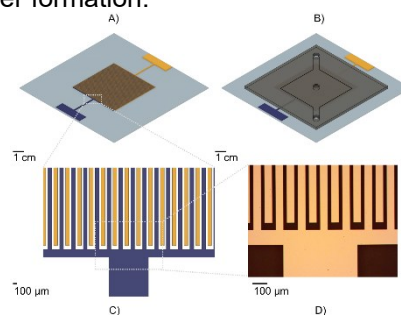


Fig. 3: IDE sensor for PDMS-COC device

- A) 3D CAD design, B) Integration with device,
- C) Magnified view of 50 μm -spaced patterned lines,
- D) Micrograph of final sensor (Bright-field, 5x magnification)

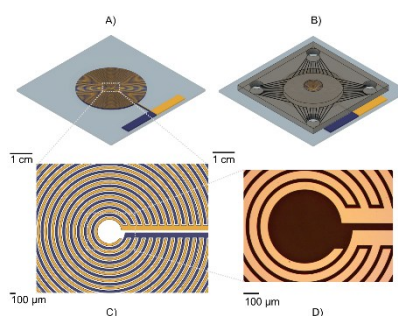


Fig. 4: IDE sensor for COC device

- A) 3D CAD design, B) Integration with device,
- C) Magnified view of 50 μm -spaced patterned lines,
- D) Micrograph of final sensor (Bright-field, 5x magnification)

Overall, this work advances the state of the art by providing a modular, reproducible, physiologically relevant system combining oxygen gradient control and real-time sensing, supporting advanced studies in next-generation OoC systems, particularly for epithelial barrier function and host-pathogen interactions.

References

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