Development of an advanced nasopharyngeal organ-on-chip system with accurate airflow patterns for the study of airborne bacterial infections.

Sophie Oatley[1,2], Michael Connor[1], Samy Gobaa[2]

1) Chromatin and Infection Unit, Institut Pasteur, Paris, FR. 2) Biomaterials and Microfluidics Core Facility, Institut Pasteur, Paris, FR

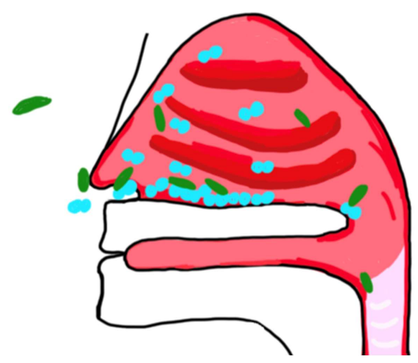
Abstract

Studying the infection processes of aerosolised pathogens is extremely challenging due in large part to the difficulty in accurately modelling the respiratory tract in vitro. Previously developed in vitro models lacked the integration of important parameters such as aerosolization, air-liquid interface, bidirectional airflow and accurate turbinate specific geometry. Our objective is to develop a physiological nasal *in vitro* device that models bacterial infection at the nasal barrier. We used 3D printing techniques with biocompatible resin, allowing for a low cost, customisable system that can capture specific aspects of the *in vivo* nasal passage. We numerically modelled and then microfabricated multiple nasal turbinate geometries to produce sub-millimetre scale airflow patterns. We ensured that the produced microfluidic device is compatible with cell perfusion and live cell imaging. Our device is advantageous over commercial systems (Transwell and Emulate technology) as it allows accurate modelling of the complex airflow patterns in the nasal passage, it can be customised at low cost, and it has the capacity for many devices to run in parallel for high throughput testing. We are using our OoC system for comparative bacterial challenge studies, which compare pneumococcal isolates of varying disease and carriage potential as well as the nasal commensal *Corynebacterium accolens* to determine the molecular processes that shape the nasal barrier – bacterial interface and their effect on bacterial transmigration, shedding and tissue level inflammatory response.

Respiration is a gateway to disease → nasal epithelial cells first contact

Respiratory infection via the nasal passage:
- Primary inhalation site of both commensal and invasive pathogens
- Often obligate initial colonization site for respiratory pathogens

Nasal complexity poorly replicated *in vitro*:
- Lack of bidirectional airflow
- Lack of physiological microenvironment
- Lack of aerosolized pathogens

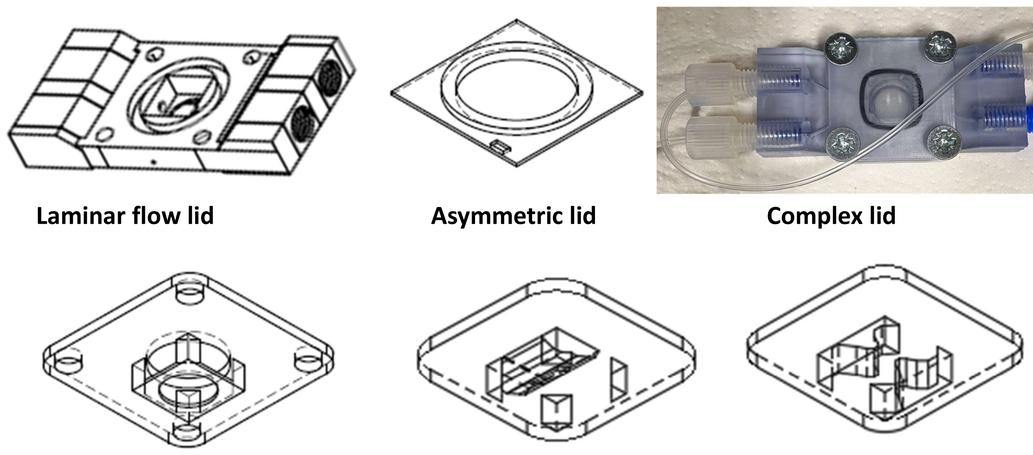


Our investigations aim to:

1) Study the impact of airflow patterns on bacterial pathogen and commensal colonization and nasal-microbe crosstalk.
2) Develop and standardize OoC device for continuous data collection sensors

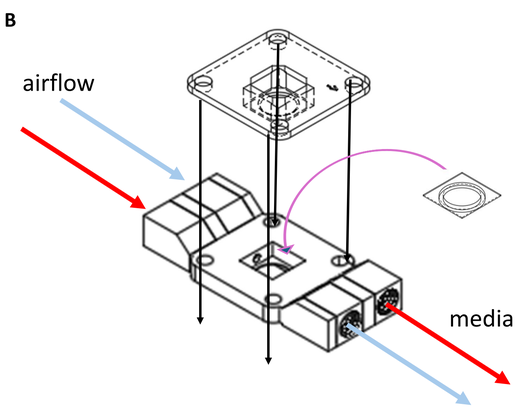
A modular nasal organ-on-chip with accurate airflow

A



A purpose built, standardized, nasal microdevice was made specifically for infection biology research in the URT.

B



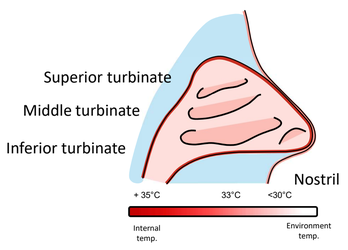
C

A) The nasal OoC device parts, constructed device, and three possible lids to create different airflows on-chip, B) the mode of construction of the nasal device, C) resin printed device for higher throughput of perfusable inserts, using interference fit with soft printed insert holder.

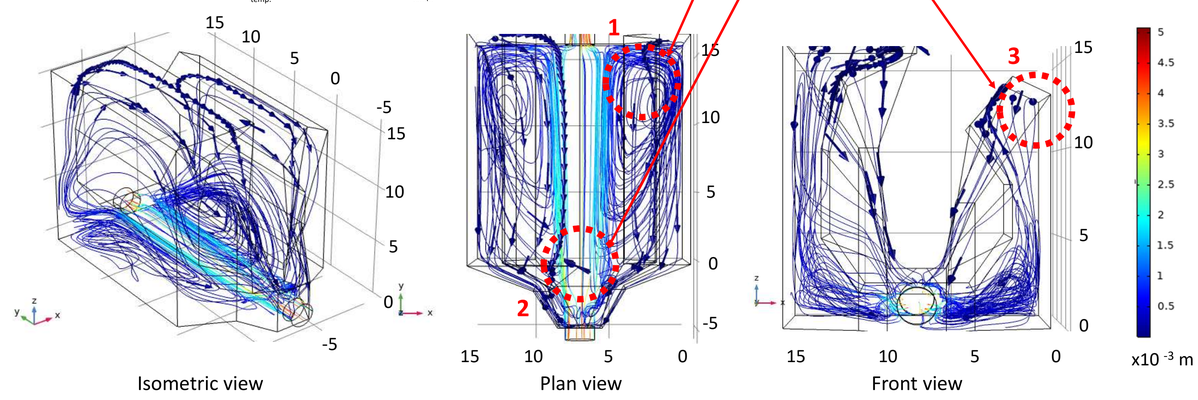
A separate liquid-liquid maturation device increases throughput and removes interdependencies between cell seeding and experimentation.

Identifying airflow regions in the nose using finite element modelling

Nasal geometry → **Simulated airflow in the nose**



Airflow types:
1. Laminar flow with slow x-y recirculation
2. Fast, laminar flow
3. Vertical, recirculating flow



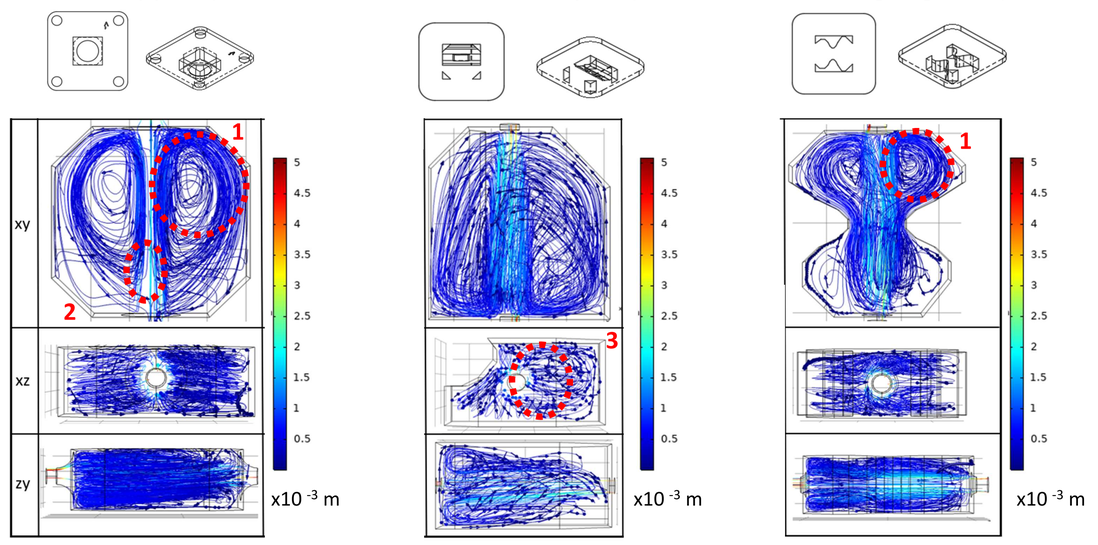
Nasal passage simulations identified three distinct flow patterns of interest for infection.

Replicating airflow patterns on-chip using modular lids

Laminar flow
(with slow loops forming in the x-y plane)

Asymmetric
(with slow loops forming in the vertical plane)

Complex multi-flows
(multiple different flow types including stagnation)

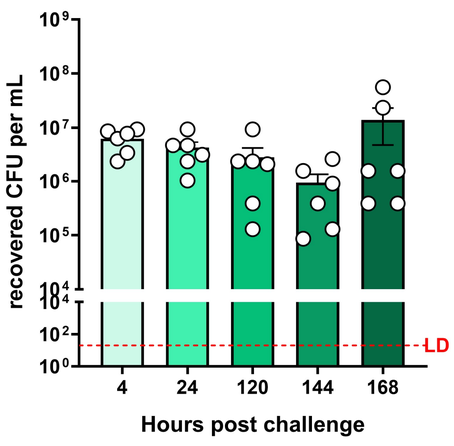


The three airflow regions were replicated on-chip using different lid geometries.

Does corynebacterium stably colonise human nasal cells?

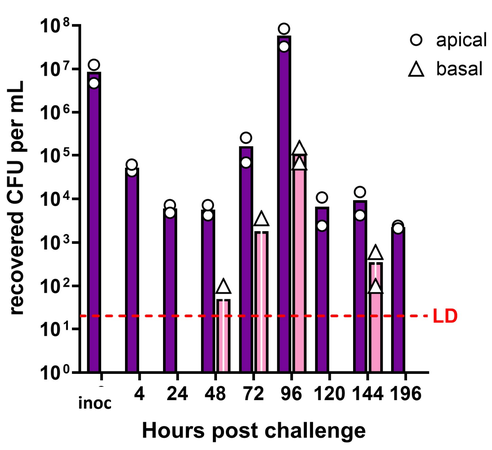
A

Coryne stably colonises undifferentiated nasal cells



B

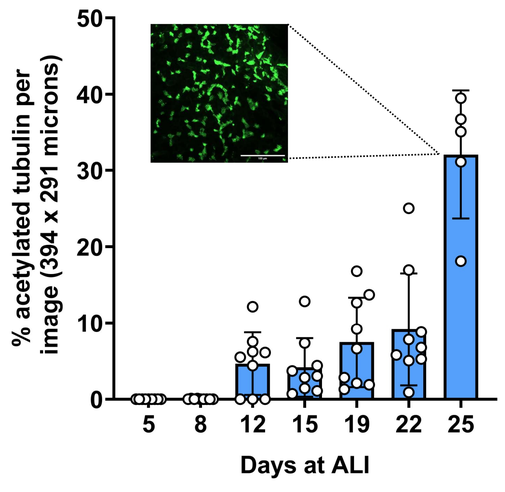
Coryne colonises differentiated human nasal cells at ALI



- ***Corynebacterium accolens*** **colonises both differentiated and undifferentiated human primary nasal cells out to 7 days.**
- **Primary nasal cells mature at ALI under static conditions, reaching a maturity plateau at around 22 days, indicating the potential infection window**

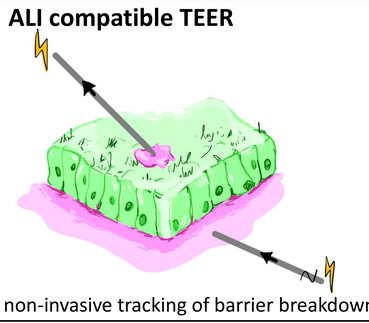
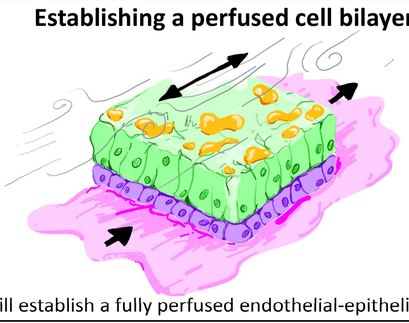
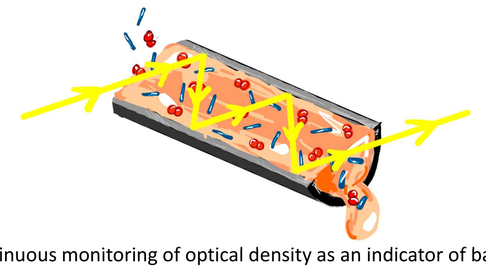
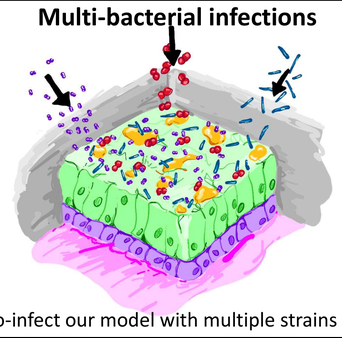
C

Characterisation of maturation of nasal epithelium at ALI



A) *Corynebacterium accolens* stably colonises HPNECs grown (undifferentiated) under submerged conditions out to 7 days, B) *corynebacterium accolens* colonises HPNECs matured at ALI in the static Transwell condition with some basal bacteria found from 2 days onwards, C) Maturation of HPNECs at ALI for 25 days with maturation quantified using coverage of acetylated tubulin.

Future directions

Continuous Sensors	Biology
ALI compatible TEER  Time-resolved, non-invasive tracking of barrier breakdown.	**Establishing a perfused cell bilayer**  We will establish a fully perfused endothelial-epithelial bilayer.
Optical density  Continuous monitoring of optical density as an indicator of bacterial burden using optical fibres.	**Multi-bacterial infections**  We will co-infect our model with multiple strains of bacteria.

References and acknowledgements:

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CommensalNose ANR JCJC

References:
[1] Kiao Inthavong,. Et al,. 2019, 'Geometry and airflow dynamics analysis in the nasal cavity during inhalation', Clinical Biomechanics, Volume 66