CEPR
Centre d'étude des pathologies respiratoires

université de TOURS

Inter-individual variability in human primary nasal cells to evaluate new nasally inhaled treatments.



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Inserm
La science pour la santé
From science to health

Mike Pasteur

AERO drug

BIONOSE

Background | The intranasal route

Intranasal delivery represents an effective method for delivering treatments directly to the nasal mucosa as inhaled particles

Advantages

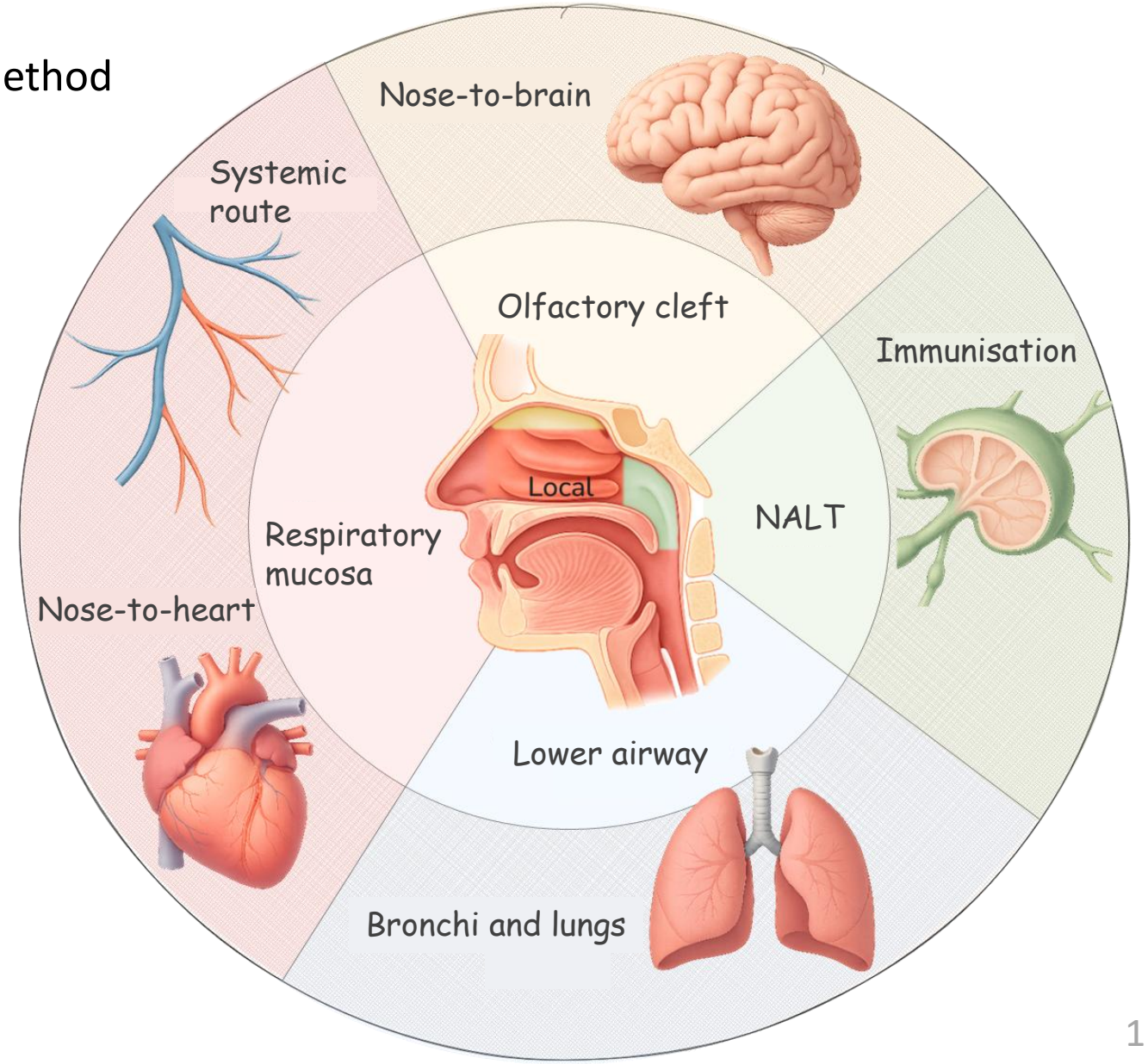
- Local delivery
- Rapid onset
- Patient convenience
- Fewer side effects than IV route

Main local indications

- Nasal allergies
- Chronic rhinosinusitis
- Nasal polyps

Other targets…

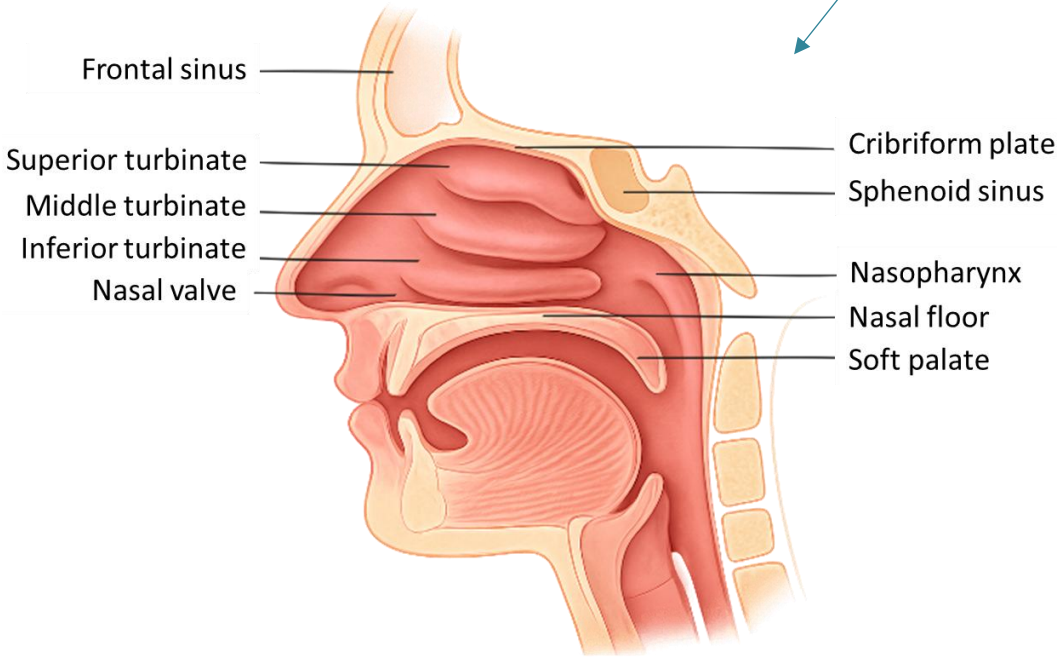
➢ Needs of precise *in vitro* studies



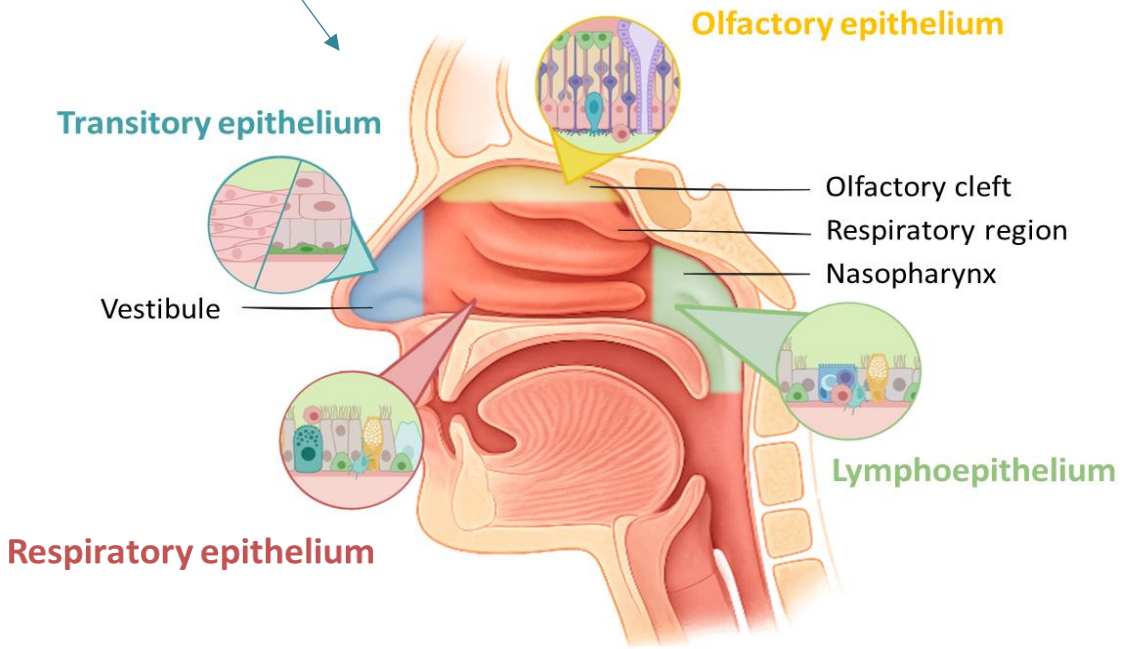
Background | The complexity of nasal cavity influencing treatment efficacy

Factors influencing the efficacy of drugs

The nasal anatomy



The nasal epithelia



Because of

- Complexity
- Tightness
- Variations

Pasteur *et al.*, 2024

Impacting the targeting of areas and the interaction between the drug/epithelium

To investigate

Because of

- Nasal defences (enzymatic activity)
- Mucociliary activity

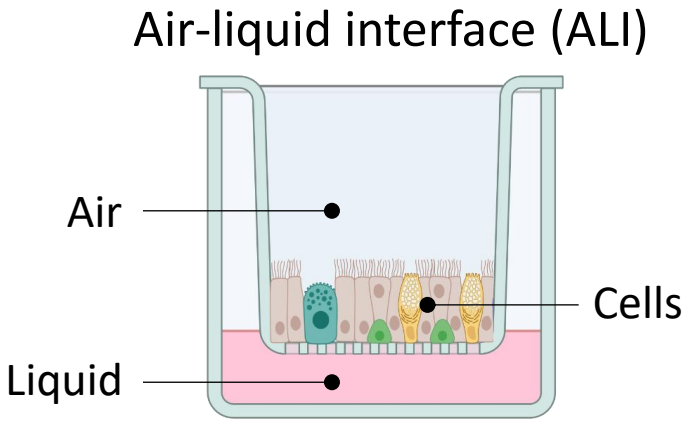
Background | Culture of respiratory epithelium at the air-liquid interface (ALI)

- Epithelial nasal cell lines (RPMI 2650)

Cost-effective / Far from the physiological epithelial reality

- Human primary nasal epithelial cells (hPNEC)

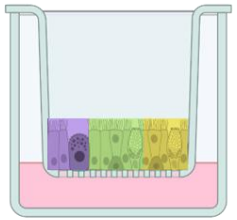
Complexity of the nasal mucosa / Expensive



Commercially available pools - widely used

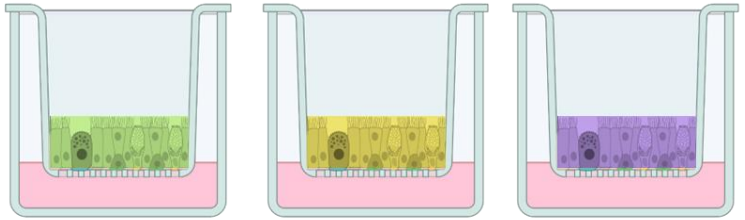
Mask inter-individual heterogeneity

Loss of statistical power



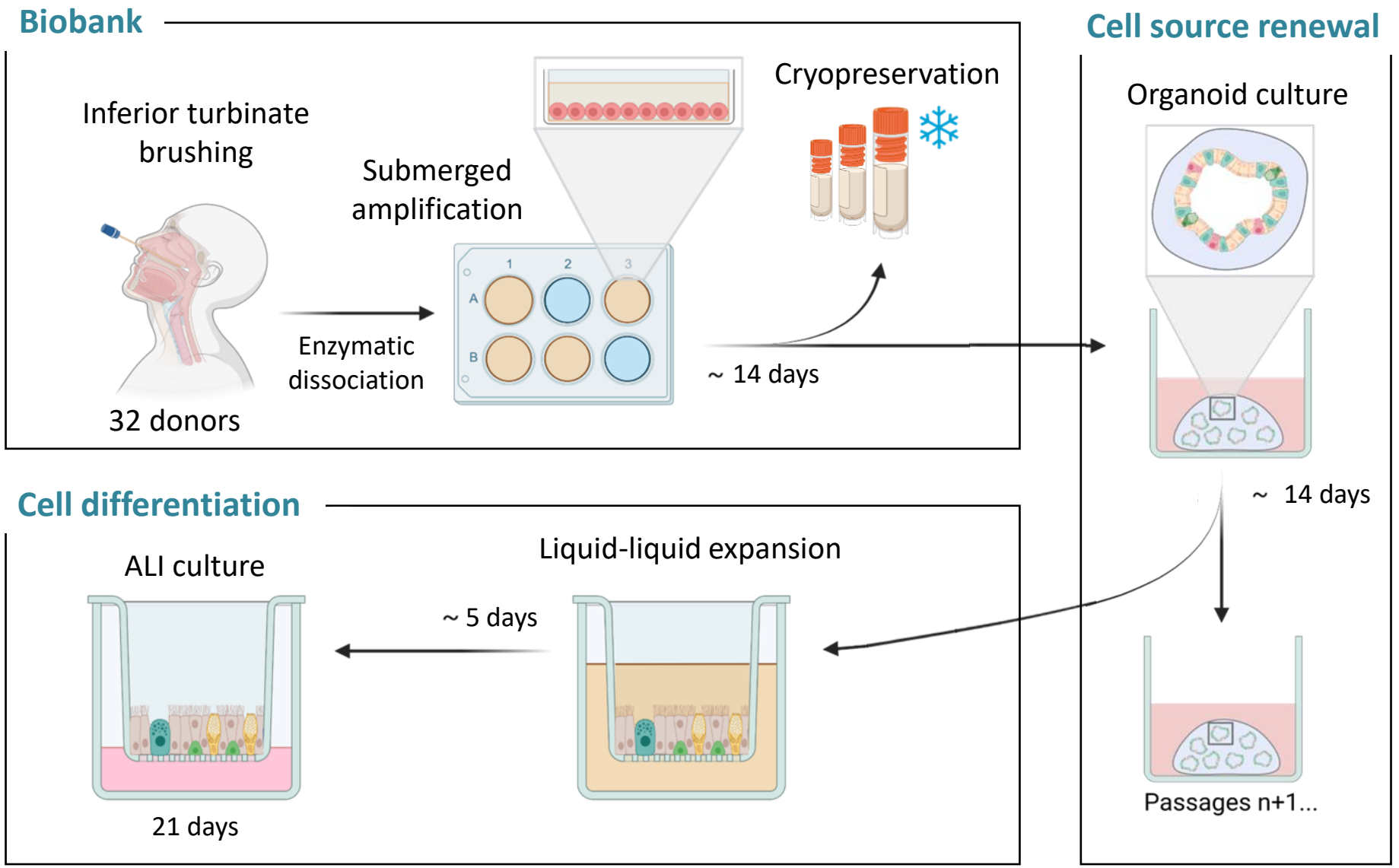
Individual cultures from nasal brushing

Time consuming, ethical regulations



➢ **We hypothesized that variation in hPNEC could have a potential impact on pathologies and intranasal treatments.**

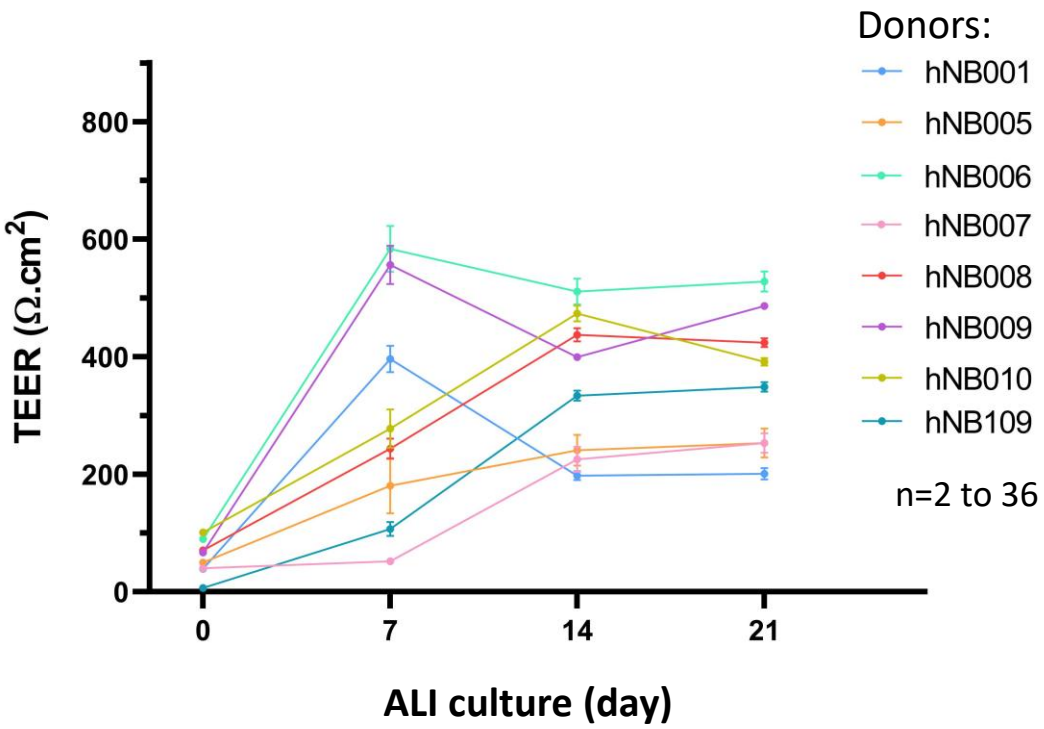
Results | 1. Optimisation of cultures and biobank creation



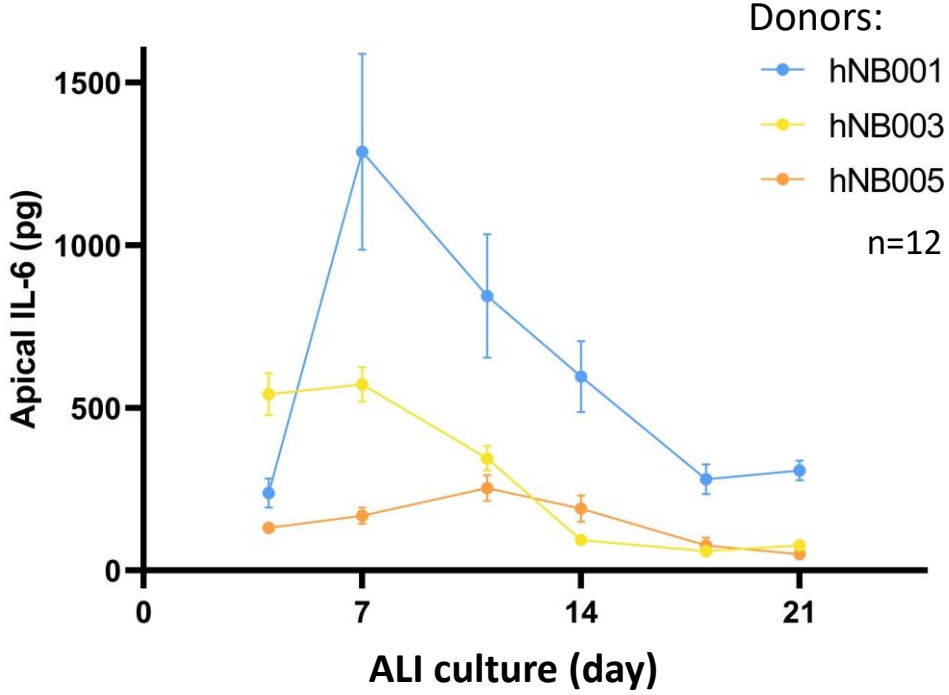
Results | 2. Epithelial characterisation: TEER and inflammatory cytokine



TEER: Trans-epithelial electrical resistance



Basal inflammatory state



- We found a high variability in epithelial formation between donors
- Profiles are indicating airway basal cell proliferation kinetic, as expected

Results | 2. Epithelial characterisation: structure and functions

Immunofluorescence staining

Ciliary cell

Ac α-Tubuline

Hoechst



Goblet cell

MUC5AC

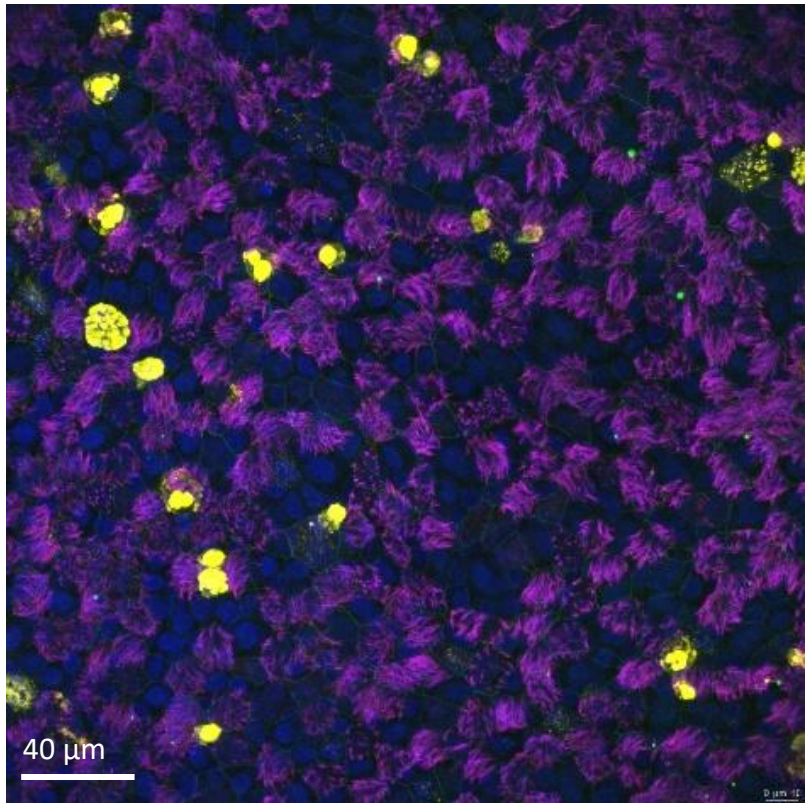


Basal cell

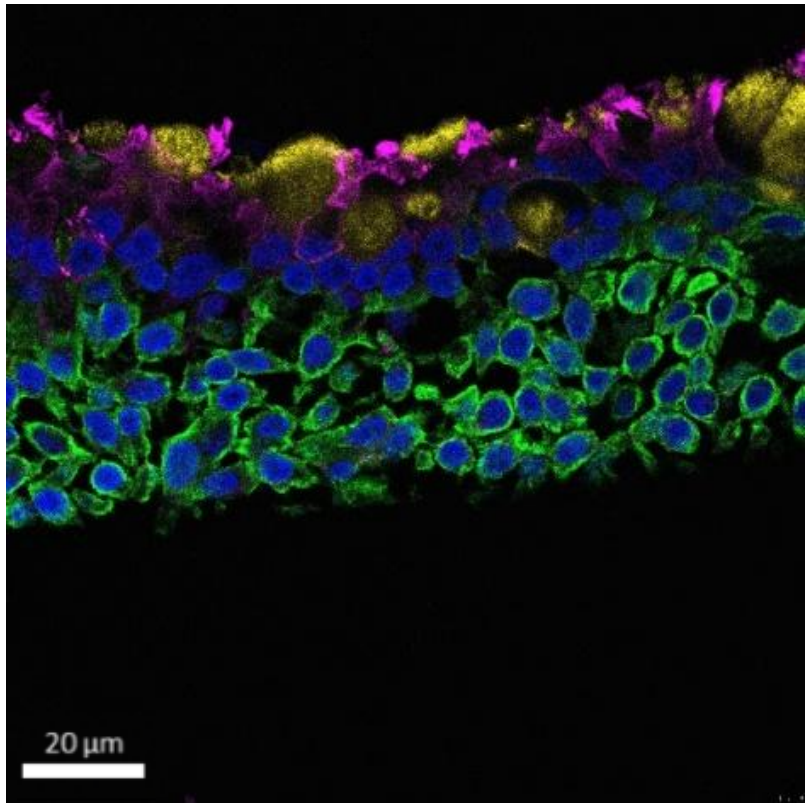
KRT5



Top view



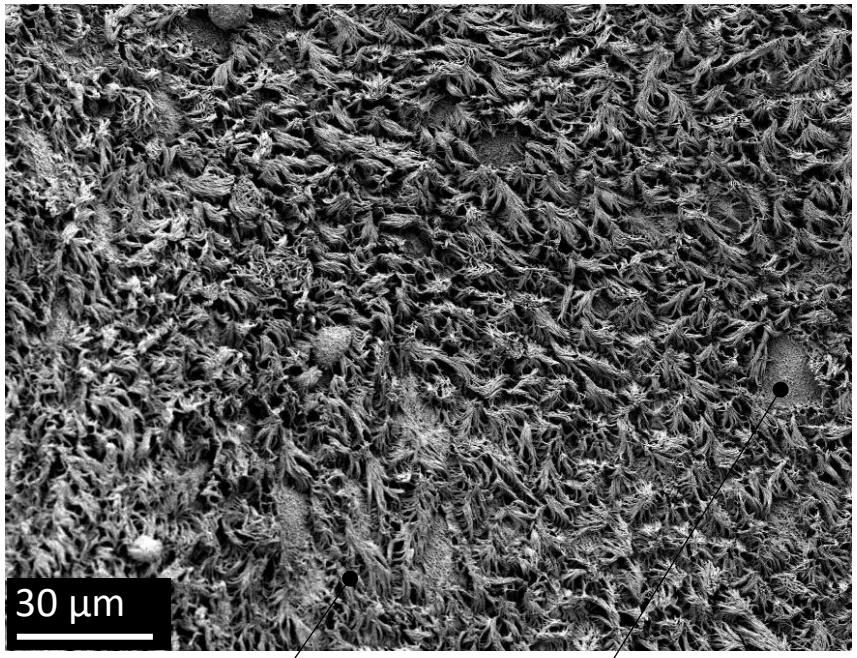
Cross-sectional view

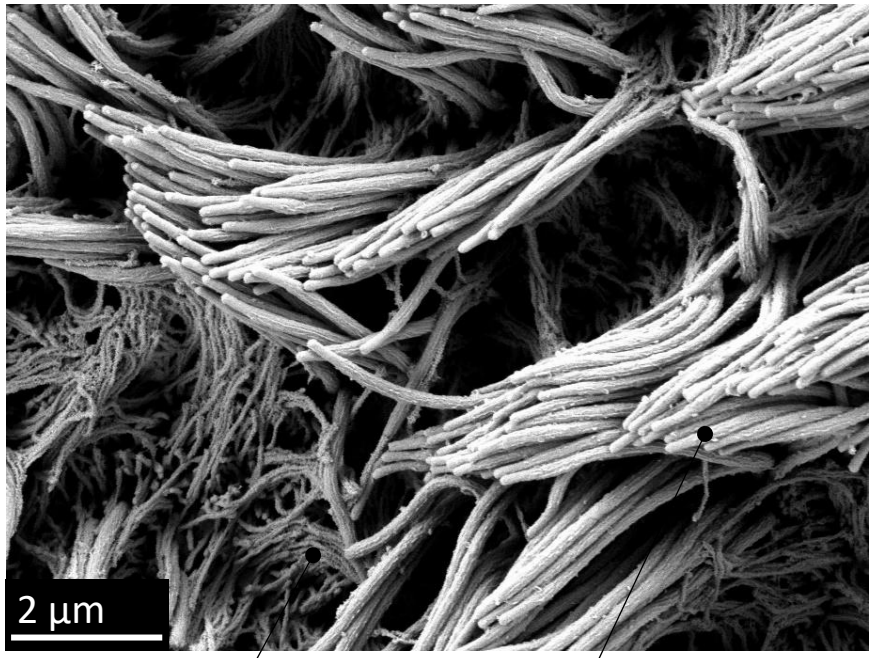


➢ We observed the main cell types of the respiratory epithelium, with different proportions depending on the donors

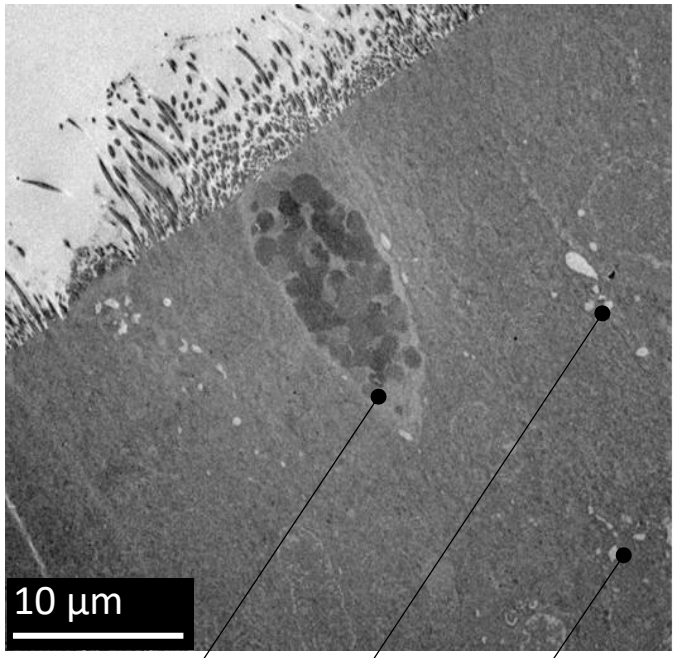
Results | 2. Epithelial characterisation: structure and functions

Scanning Electron Microscopy



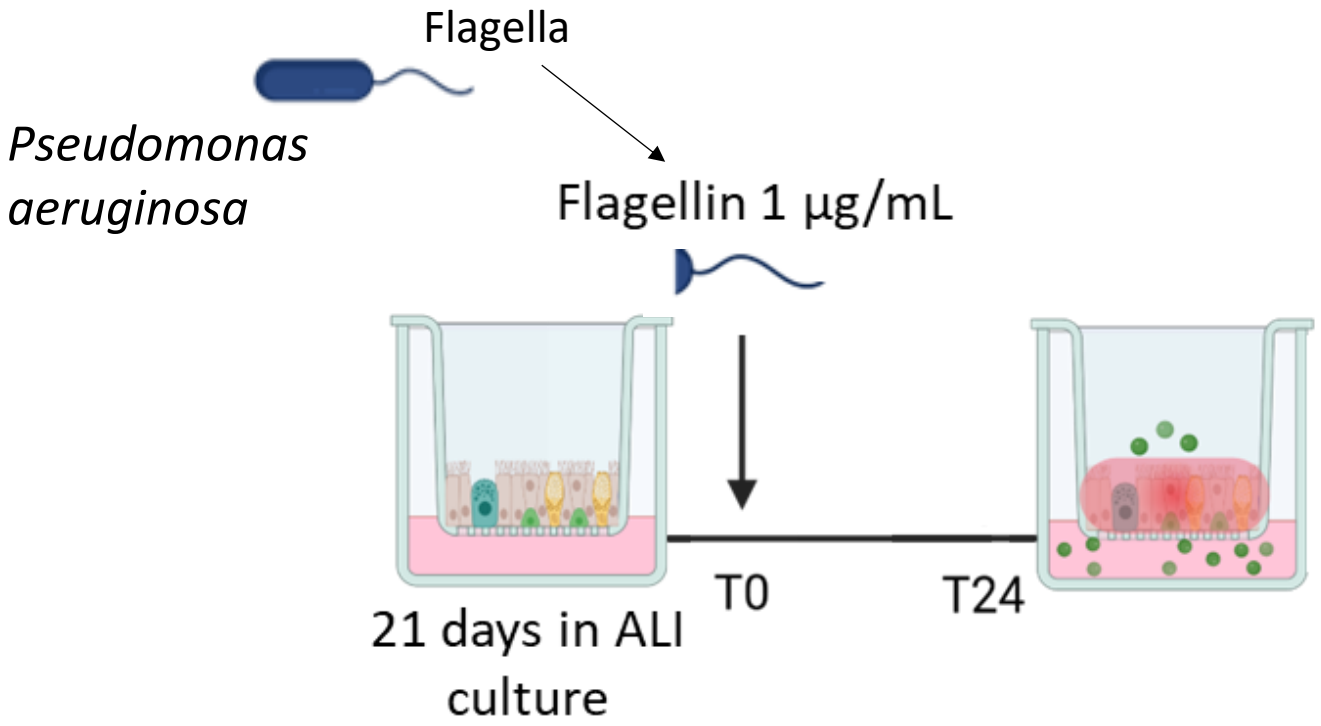


Transmission Electron Microscopy



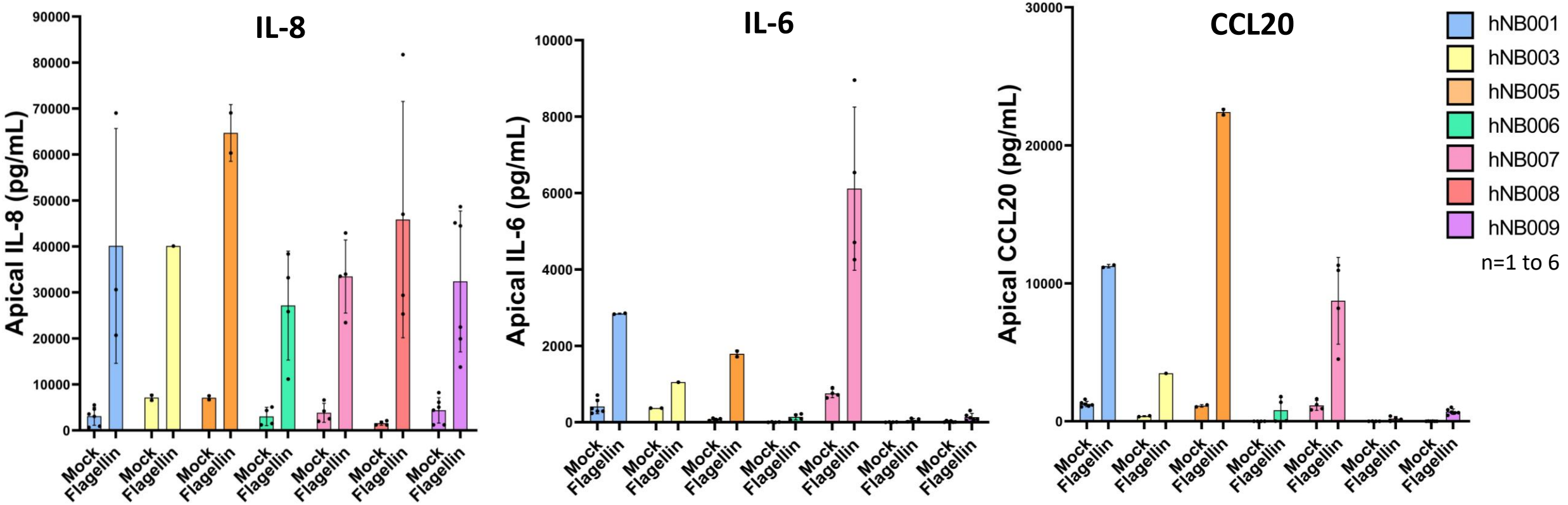
➢ Full differentiation: success rate of 95 %, higher than reported in other studies.

Inflammatory model mimicking bacterial infection



- Pro-inflammatory cytokines
- Epithelial integrity (TEER)
- Viability

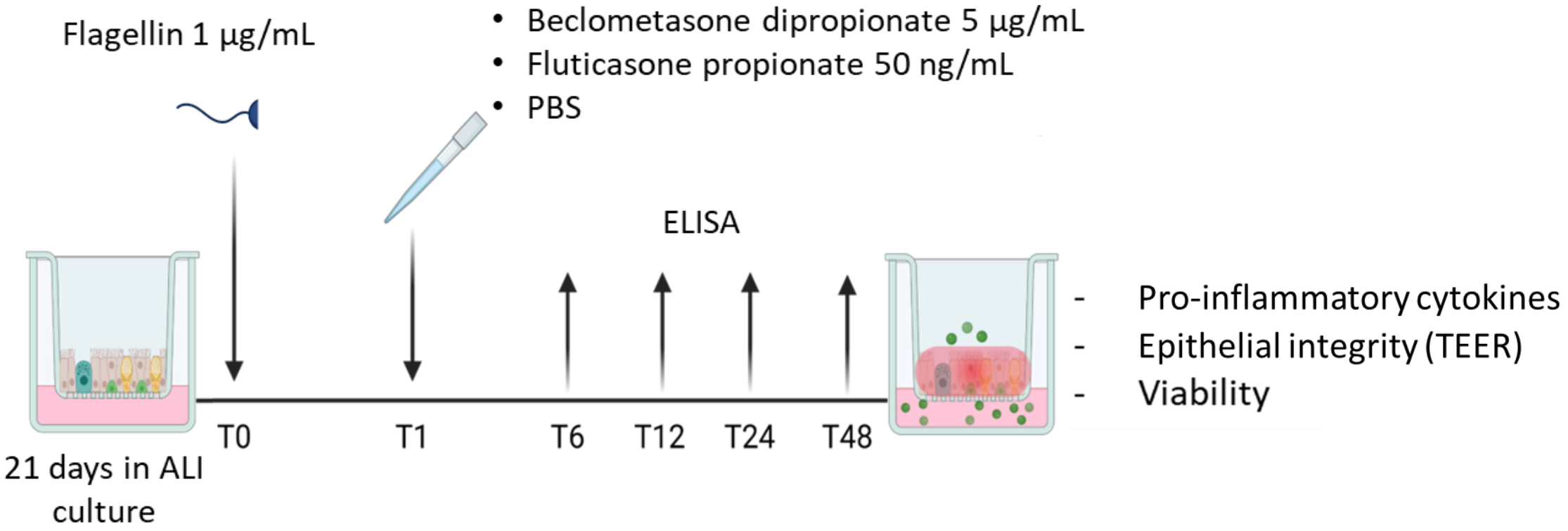
Results | 3. Variability of cellular response in an inflammatory context



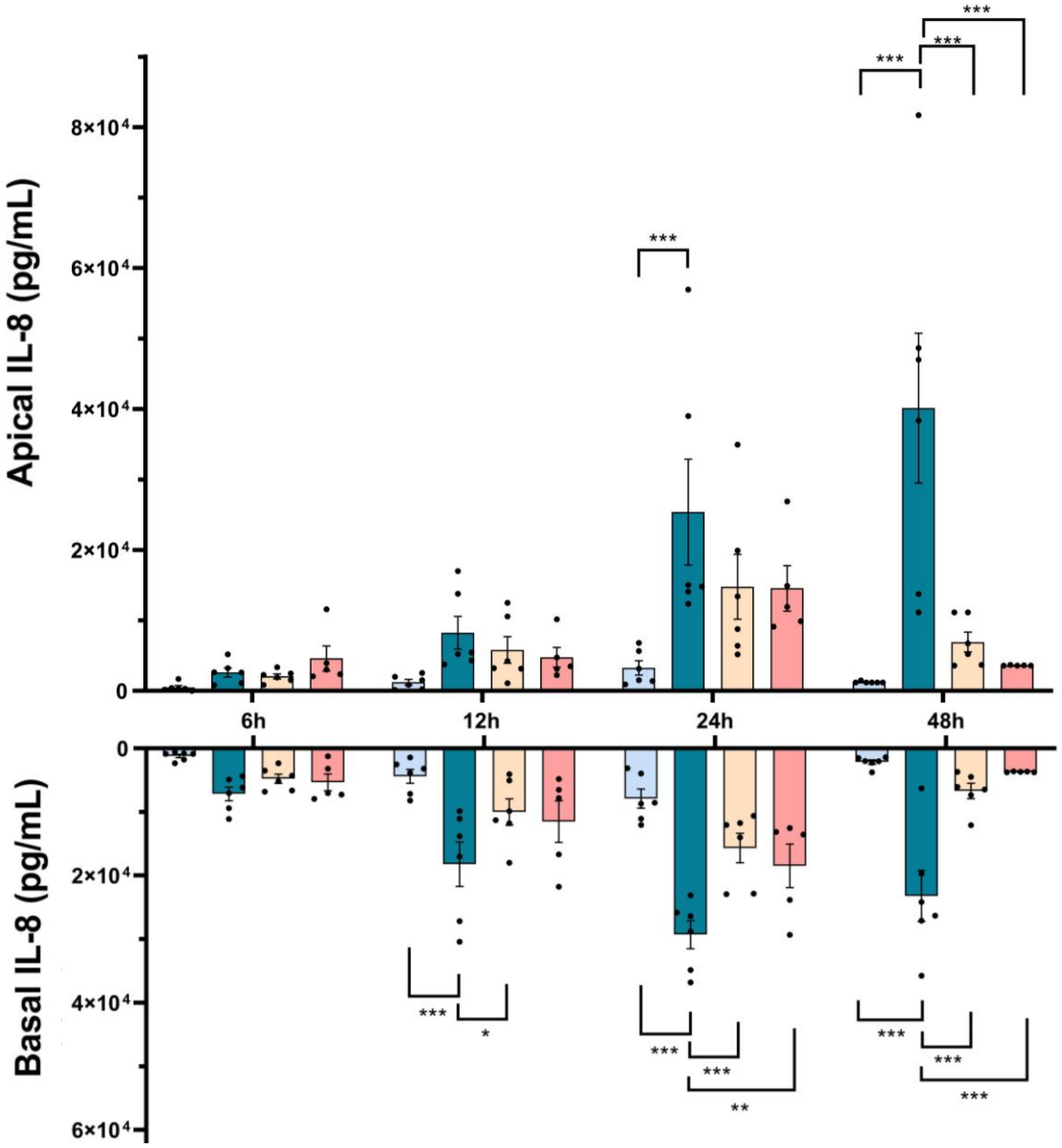
- Evidence of high inter-individual heterogeneity in the basal state and in the inflammatory context
- The observed differences are probably due to the individual cell sub-type proportions and epigenetics since TLR5 expression is not correlated to cytokines expression

Results | 4. Validation by use of anti-inflammatory drugs

Effect of commercial anti-inflammatory drugs (intranasal route)



Results | 4. Inflamed hPNEC in ALI culture as a model for drugs testing



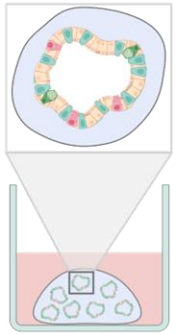
Kruskal Wallis test, 3 donors, n=2 per donors

- Mock
- Flagellin + PBS
- Flagellin + Beclometasone Dipropionate
- Flagellin + Fluticasone Propionate

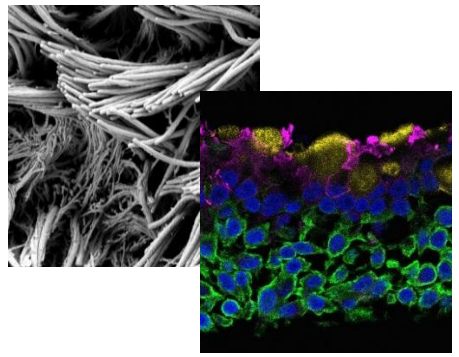
- We observed a significant reduction of the IL-8 inflammation with corticoids, without affecting the epithelial barrier
- This method successfully established a representative *in vitro* model of the nasal mucosa drug studies

- What about IL-6/CCL20?
- Is this applicable to all donors?

Conclusion | hPNECs as model for pathology studies and drug testing?



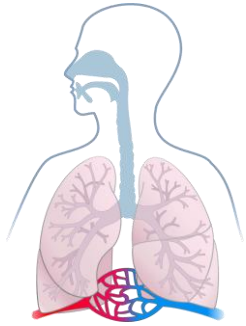
- Our culture method successfully produced well-differentiated hPNEC
- Our study highlights the *in vitro* heterogeneity present in the nasal mucosa of healthy individuals



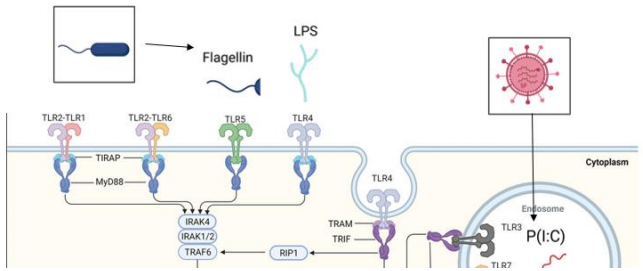
- Creation of a large biobank and inflammatory model for future inter-individual studies
  - ➢ Robust platform for evaluating new inhaled therapies in ALI cultures.
  - ➢ Facilitate the systematic investigation of patient-specific variability in drug response.

Opportunities

Personalized medicine



Host-pathogen interactions



Therapeutics studies (aerosol compatible)



CEPR Centre d'étude des pathologies respiratoires

Thanks for your attention!

université de TOURS



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AERO drug

BIONOSE

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From science to health

Background | The complexity of nasal cavity influencing treatment efficacy

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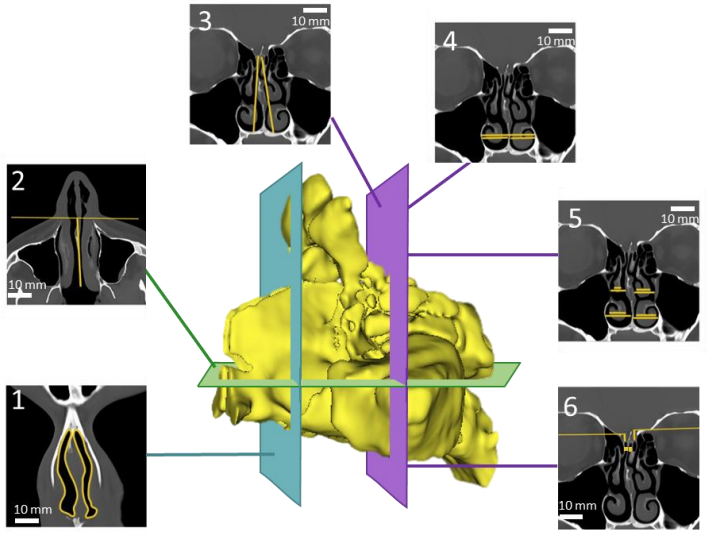
International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

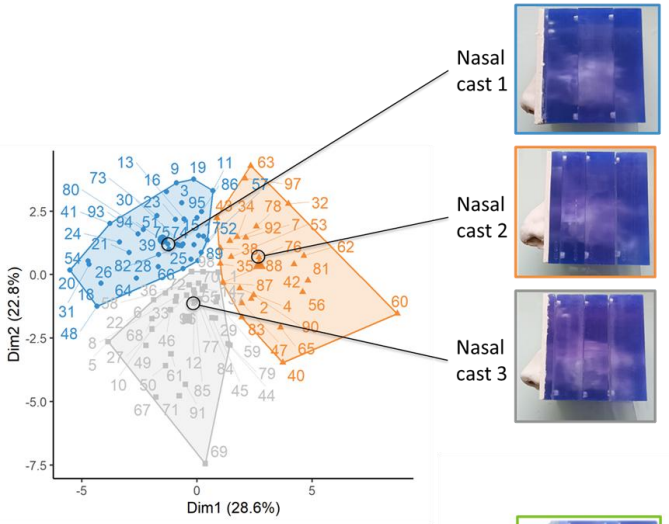
Characterization of anatomical variations of the nasal cavity in a subset of European patients and their impact on intranasal drug delivery

Mike Pasteur [a,b,1], Guillaume Arsouze [c,1], Guy Ilango [a,b], Déborah Le Pennec [a,b], Dimitri Kulker [d], Anaïs Heyraud [a,b], Jean-Philippe Cottier [c], Charles Aussedat [a,e], Nathalie Heuzé-Vourc'h [a,b], Virginie Hervé [a,b,2,*], Sandrine Le Guellec [a,b,f,g,2,*]

2D/3D measurements



Clustering and 3D printing



Nasal cast 1

Nasal cast 2

Nasal cast 3

Published model
Derivated from a plastinated head

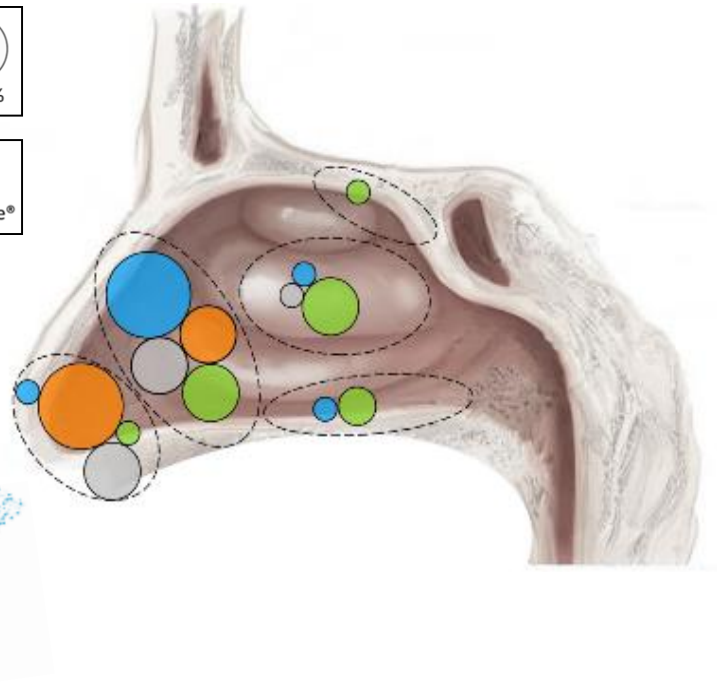
Cabrera *et al.*, 2017;
Le Guellec *et al.*, 2021

Aeronose®

Deposition testing

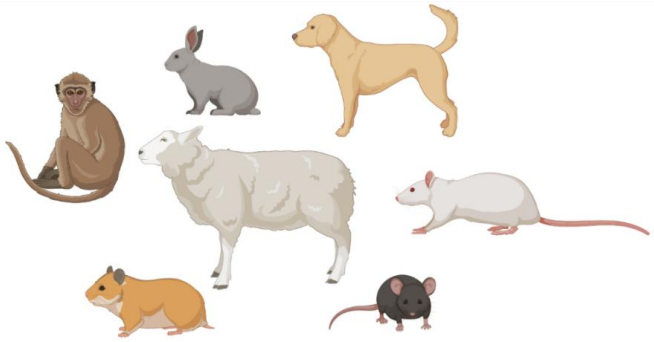
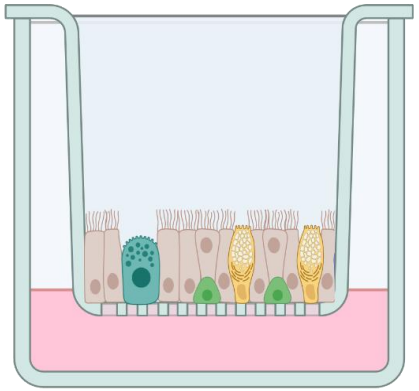
Fluoresceine deposition: 1-5 % | 6 – 25 % | 26 – 50 % | > 50 %

Nasal cast: NC 1 | NC 2 | NC 3 | Aeronose®



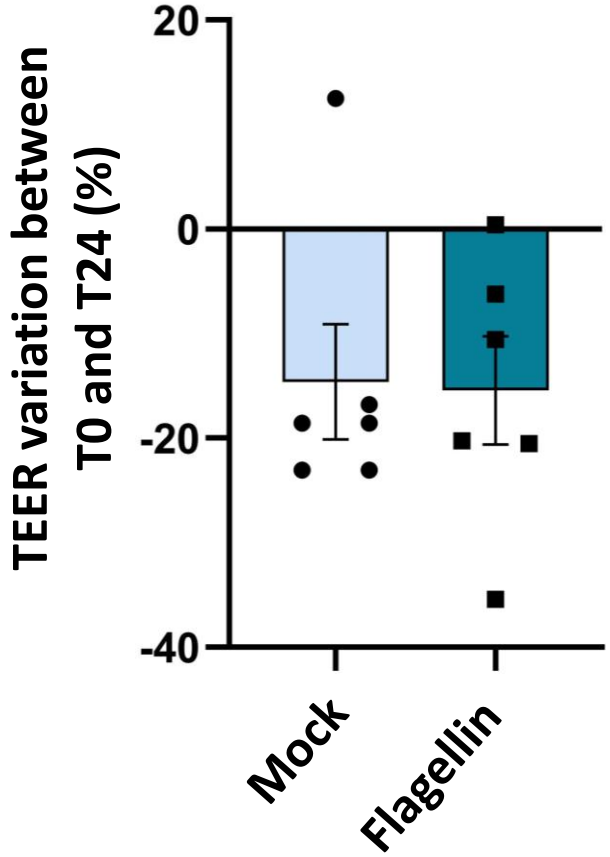
- Individual anatomical variations significantly affect intranasal deposition

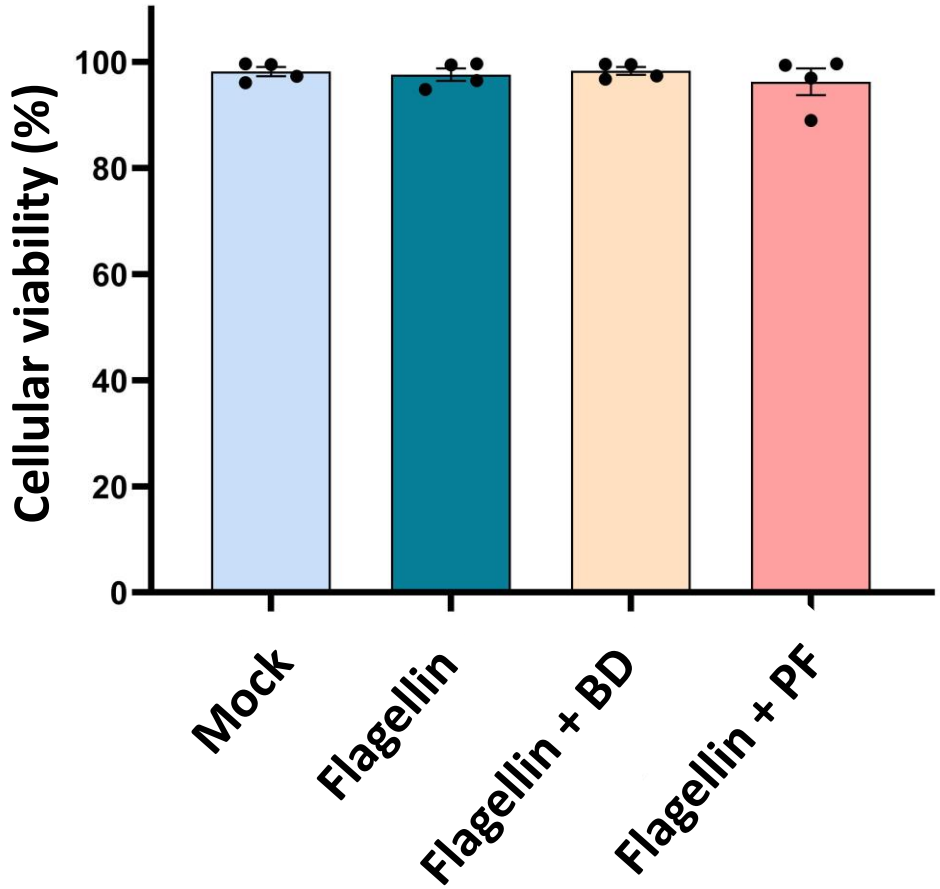
Background | Experimental models for inhaled drugs

	In vivo models	Cellular models	Anatomical models
			 Le Guellec *et al.*, 2014
Applications	Toxicology Pharmacokinetics Pharmacodynamics	Screening Toxicology Pharmacodynamics	Intranasal deposition
Limits	≠ devices to clinic ≠ anatomy to humans ≠ physiology to humans	No deposition data Do not recapitulate the realistic micro-environment	Inert, only t=0 prediction Do not take anatomical variability into account

➢ What about the representativeness of models?

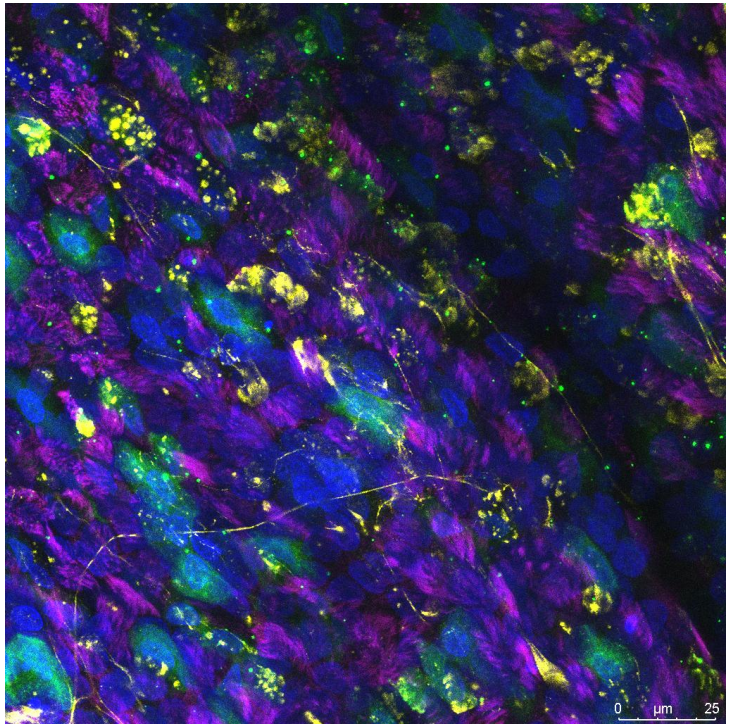
Results | TEER variation and cell viability



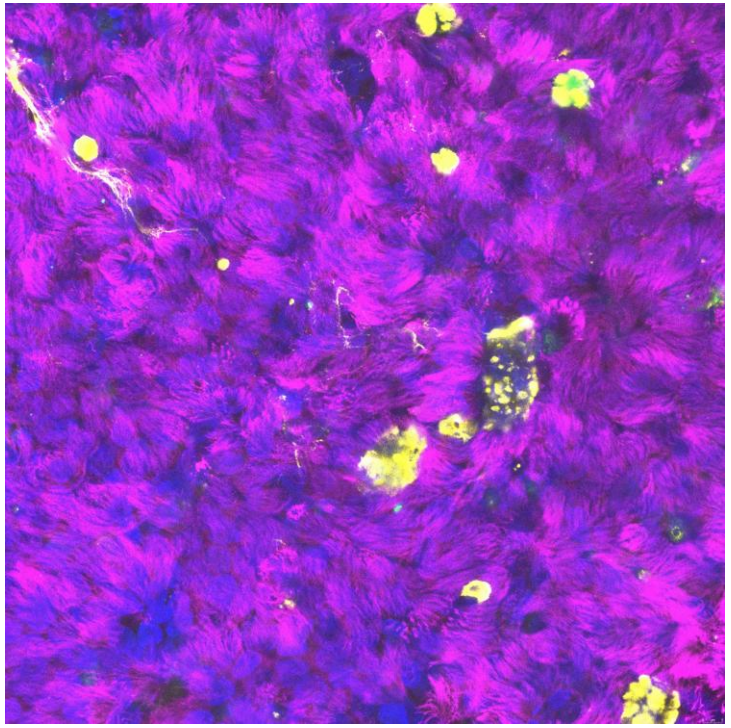


Results | Immunostaining for different donors

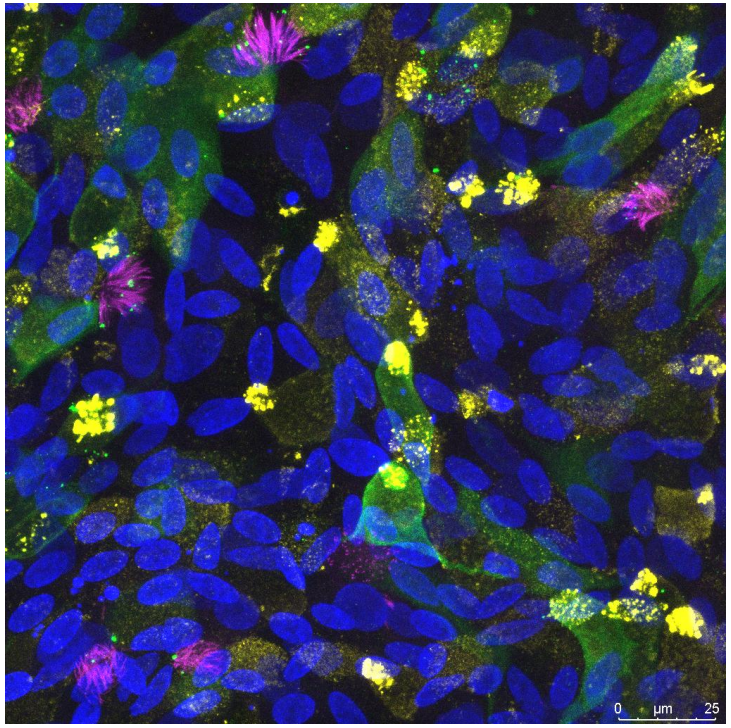
hNB005



hNB008



hNB009



➢ Differences in cell sub-population between donors

- We will characterise these variations