Using airway organoids and Air Liquid Interface cultures to profile the spatial and temporal dynamics of *P aeruginosa* infections

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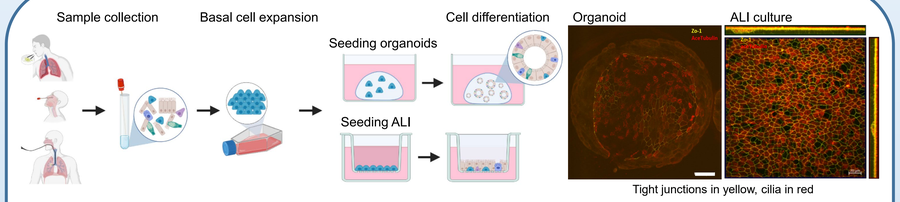
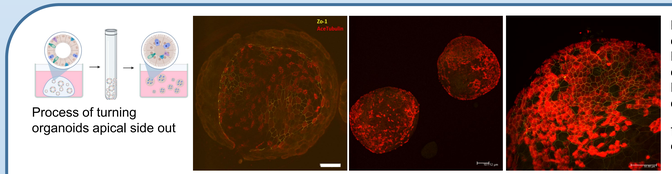
Thick mucus that builds up in the lungs of cystic fibrosis (CF) patients offer a favorable colonization environment for opportunistic pathogens like *Pseudomonas aeruginosa*. *P. aeruginosa* typically become the predominant organism in CF lungs, and chronic infection is linked to progressive decline in pulmonary function and early death. With this project we address why some infections persist, if we can identify bacterial and host tissue markers, indicative of prolonged colonization and progression towards persistent infection and ultimately if we can improve treatments of infections in CF patients. Here I show how we use patient derived airway organoids and ALI cell cultures in combination with longitudinal libraries of clinical *P. aeruginosa* strains to characterize early colonization processes in persistent infections.



Tight junctions in yellow, cilia in red

Cell isolation and cultivation. When cultured in matrigel or at Air Liquid Interface (ALI), primary bronchiolar basal cells differentiate and self-organize into 3D pseudostratified epithelia. Basal cells can be isolated from nasal brush biopsies, lung biopsies or BAL fluids.

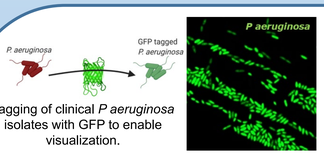


Process of turning organoids apical side out

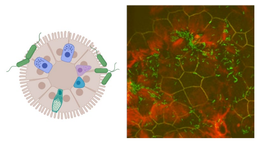
Panel 1: apical in organoid
Panel 2: apical out organoids
Panel 3: apical out organoid, zoomed in

Tight junctions in yellow, cilia in red.

Turning organoids inside out. To enable host-pathogen interactions on the apical surface of the organoids, we use suspension culturing to generate apical-out polarity in our airway organoids. Apical-out airway organoids reorganize with apical junctions and motile cilia facing the organoid exterior, thus facilitating bacterial colonization on the apical-surface.

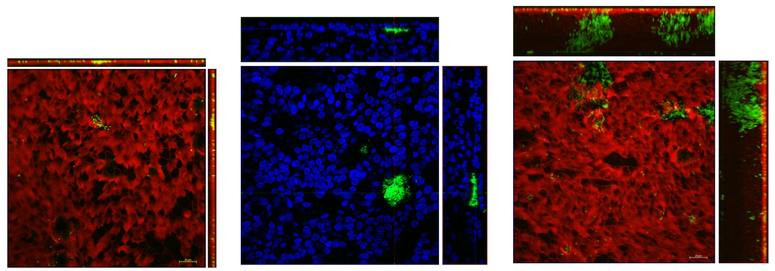


Tagging of clinical *P aeruginosa* isolates with GFP to enable visualization.



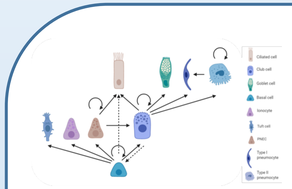
P aeruginosa colonizing an apical out organoid.

Tight junctions in yellow, cilia in red, *P. aeruginosa* in green.

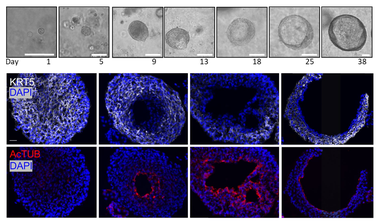


Infection timeline on ALI cultures. *P aeruginosa* in green, tight junctions in yellow, cilia in red, nuclei in blue

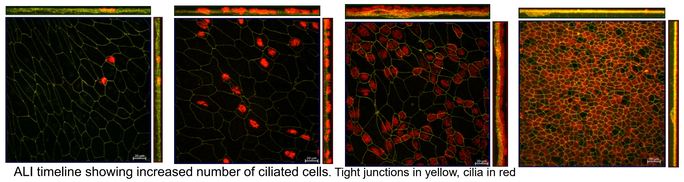
Visualizing the spatial and temporal infection. *P aeruginosa,* inoculated on the apical side of differentiated cell cultures, penetrate the epithelial layer and proliferate within the tissue. Extended co-culture results in complete destruction of the epithelium.



Basal cells can differentiate into all cells of the airway when given the right signals.

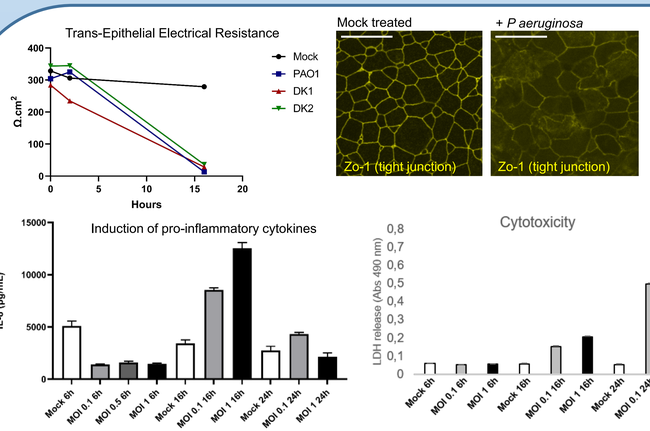


Organoid development. Basal cells in white, nuclei in blue, cilia in red

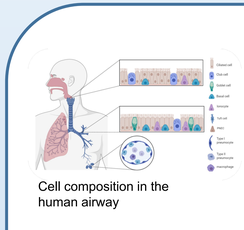


ALI timeline showing increased number of ciliated cells. Tight junctions in yellow, cilia in red

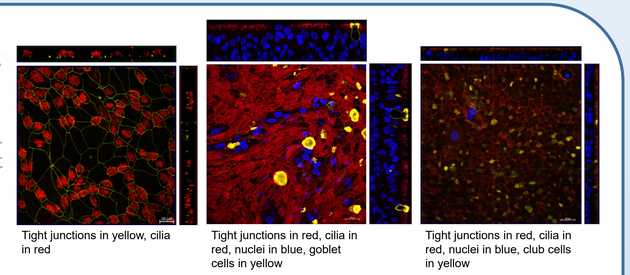
Organoid and ALI differentiation. Organoids initially form solid basal cell spheroids and will over time develop lumens with ciliated cells lining the interior surface. In both organoids and ALI we see an increase in ciliated cells, but no evidence of degradation or de-differentiation during 45 days of culture.



TEER is measured to determine the effect of *P. aeruginosa* infection on cell layer integrity. The observed time-dependent drop of TEER, indicate progressive disruption of the epithelial cell barrier function.
Host reactions to bacterial infections are analyzed (here illustrated by induction of cytokine IL-8). LDH cytotoxicity assays are performed to assess functionality and integrity of the cells during the infection.



Cell composition in the human airway

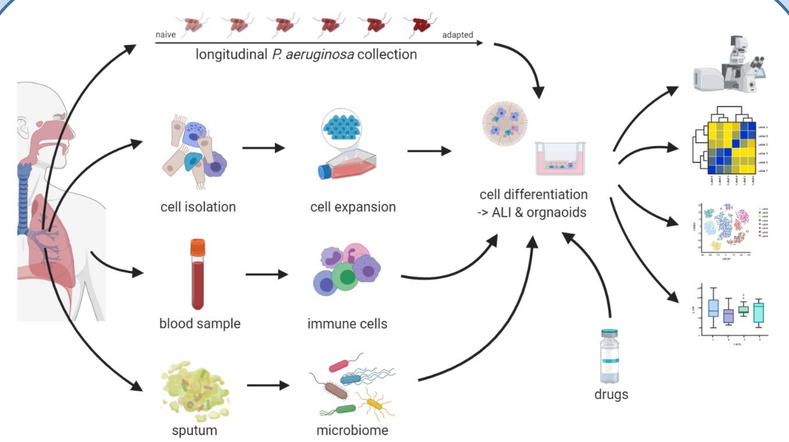


Tight junctions in yellow, cilia in red

Tight junctions in red, cilia in red, nuclei in blue, goblet cells in yellow

Tight junctions in red, cilia in red, nuclei in blue, club cells in yellow

Mimicking the airway epithelium. The major cell types normally associated with in vivo airway epithelium (i.e. basal, ciliated, secretory cells) are present within our differentiated cultures. Our cultures have normal, functioning tight junctions and maintain intact barrier function for up to 45 days of ALI culture. Thus our differentiated cultures provides an excellent mimic of the airway epithelium



Conclusions and future perspectives. Our cell models provides an excellent mimic of the airway epithelium and our infection system is a relevant platform for studying *P. aeruginosa* infections in CF. Transcriptional analysis combined with proteomics and visualization of both host and *P aeruginosa* will shed light on their intricate relationship. By combining patient derived cell cultures with longitudinal libraries of clinical *P. aeruginosa* strains we will be able to analyze how specific colonization potential and trajectories change over time in different patients and strains, and how the specific colonization is affected by adaptive geno- and phenotype changes in the bacteria.