Epithelix

***in vitro* Solutions for Respiratory Diseases and Chemical Testing**

Streptococcus pneumoniae inhibits Pseudomonas aeruginosa growth on nasal human epithelium in vitro

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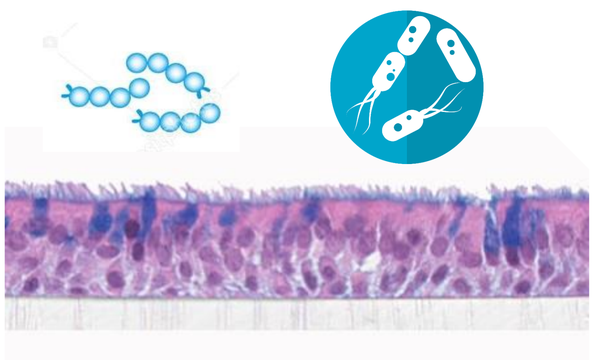
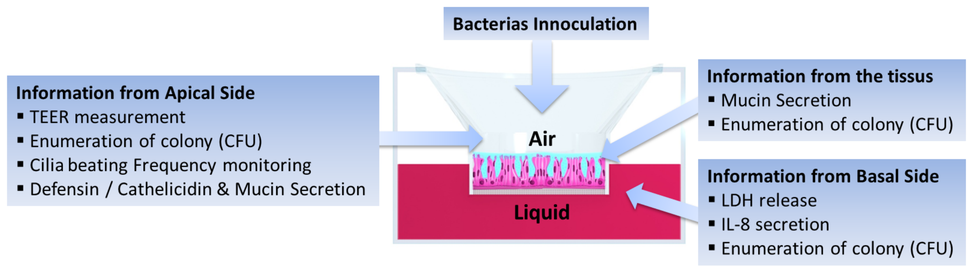
Pathogens colonizing the respiratory tract compete with a range of other bacteria. *Pseudomonas aeruginosa* (PA) infections are increasingly associated with acute exacerbations in chronic obstructive pulmonary disease. *Streptococcus pneumoniae* (SP), meanwhile is a main cause of pneumonia, meningitis, it can lead to infections and other respiratory diseases such as bronchitis.
We report herein the use of 3D airway epithelia reconstituted *in vitro* to study interactions of PA and SP on nasal mucosa. MucilAir™, a fully differentiated human airway epithelium made of a mixture of primary nasal cells from 14 donors, was used to study the effects and behaviour of PA and SP (inoculated at $3E^{+02}$ and $3E^{+11}$ CFU/cm^2 respectively) cultured separately or together over 24 hours.

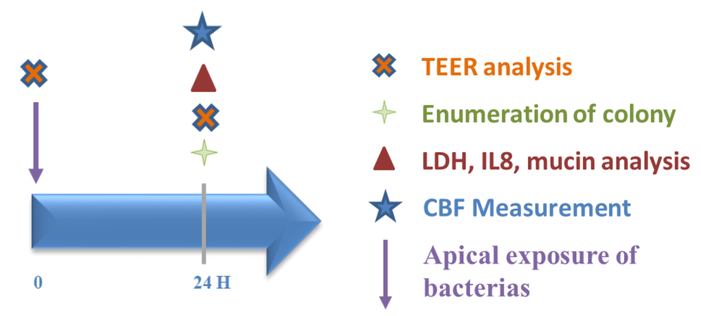
Testing Strategy



MucilAir™, a fully differentiated 3D in vitro cell model of the human airway epithelia. The cells were freshly isolated from the biopsies. After 45 days of culture at air-liquid interface, the epithelia were fully differentiated, both morphologically and functionally.

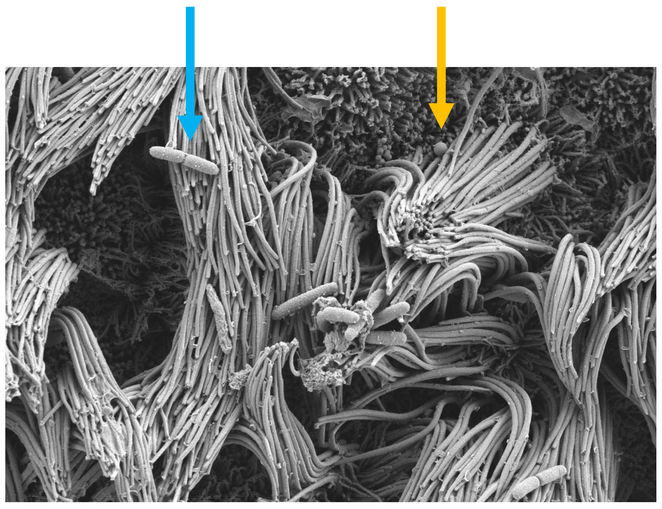


Pseudomonas Aeruginosa (PA) and Streptococcus Pneumoniae (SP) were inoculated respectively at $3E^{+02}$ and $3E^{+11}$ CFU/cm^2 on MucilAir™-pool of donors on apical side. Toxicity induced by infection is monitored by several non-destructive endpoints such as TEER measurement, cilia-beating frequency monitoring, mucin secretion, IL-8 and LDH releases

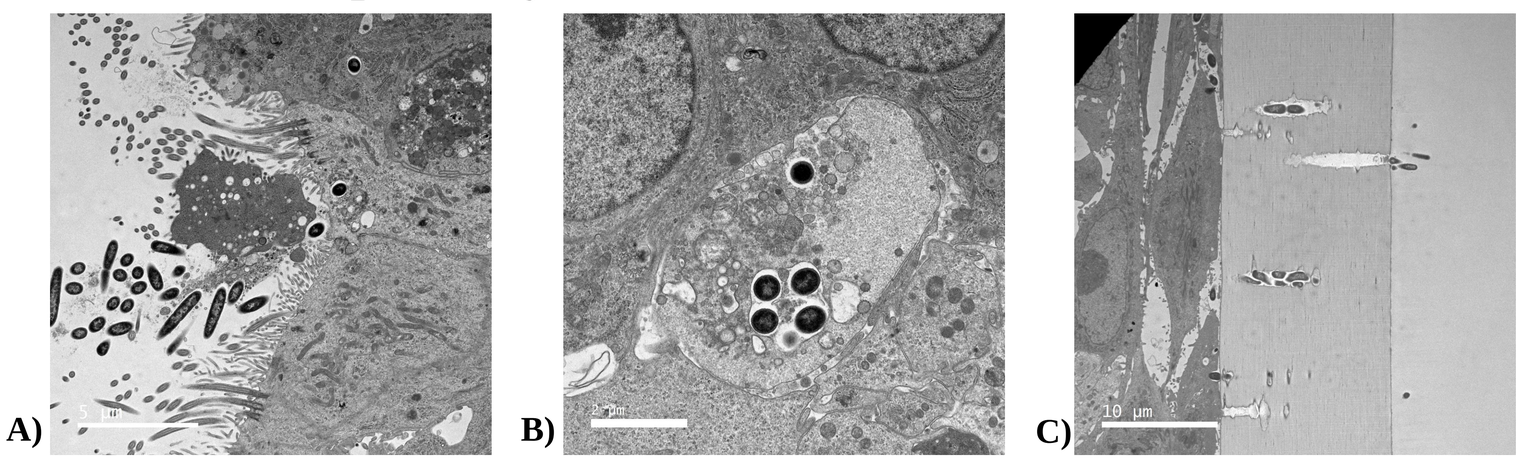


*General protocol and schedule of end point measurement, N= 3 per condition and per time point. Statistical comparison was done by Student's t test (*p<0.05).*

Microscopic analysis



Scanning electron microscopy image of *Pseudomonas aeruginosa* (blue arrow) and *Streptococcus pneumonia* (orange arrow) on MucilAir™ apical surface at 24 hours post inoculation during a concomitant infection.

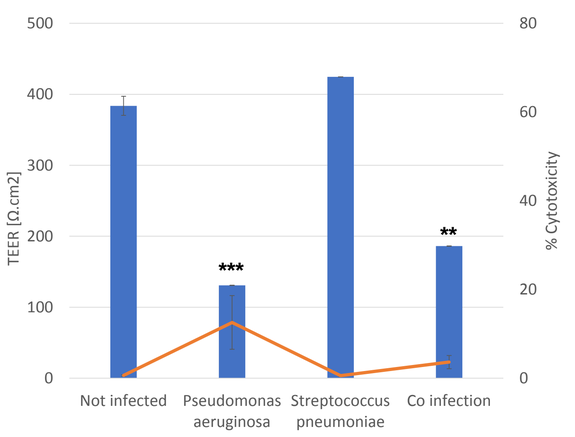


Transmission electron microscopy images representing A) internalized bacteria of *PA* and *SP* on the apical surface, B) *SP* intracellular and C) migration of PA through the pores of insert membrane

Apical, basolateral and intratissular PA and SP growth were quantified by Colony Forming Unit (CFU). Impairment of epithelial homeostatic barrier function was evaluated through monitoring the Trans Epithelial Electrical Resistance (TEER), cytotoxicity (LDH), cilia activity, mucin and IL-8 release.

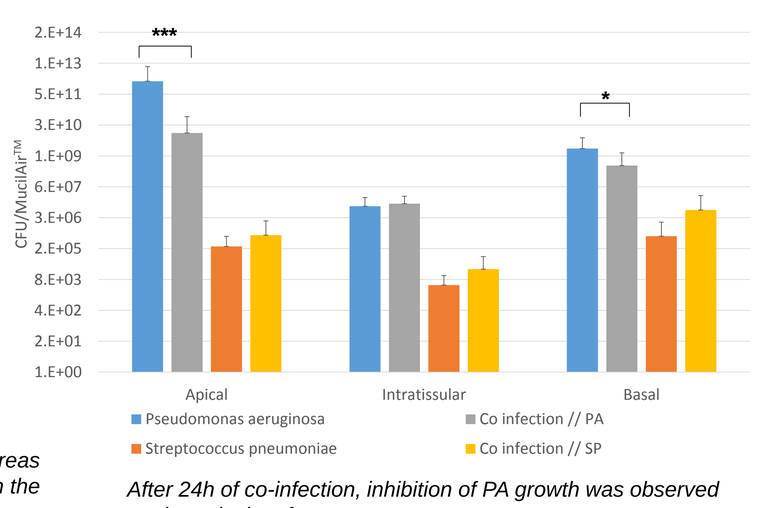
Results

Evaluation of barrier function and cytotoxicity on MucilAir™ after PA or SP infection alone or together after 24h



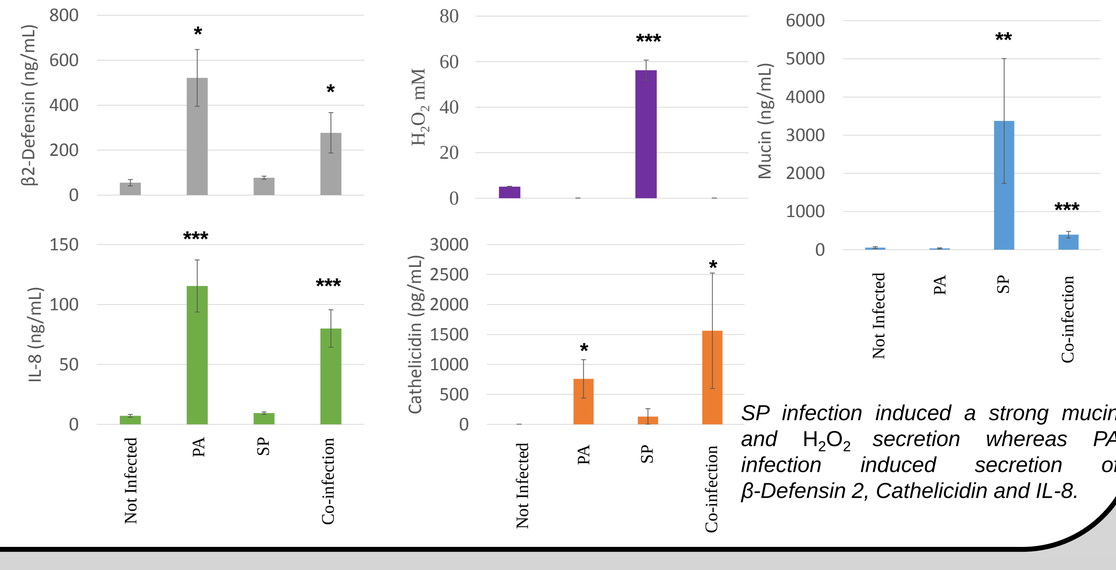
SP infection had no impact on TEER or LDH release, whereas PA induced a decrease of TEER coupled with cytoxicity. In the co-infection model, only a drop of TEER is detected.

Quantification of *PA* and *SP* growth alone or together on MucilAir™ after 24h



After 24h of co-infection, inhibition of PA growth was observed on the apical surface

Soluble factors release on MucilAir™ after PA or SP infection alone or together after 24h



SP infection induced a strong mucin and H_2O_2 secretion whereas PA infection induced secretion of β-Defensin 2, Cathelicidin and IL-8.

PA infection induces a loss of TEER, 20% cytotoxicity, an increase of Il-8, and an up regulation of β2-defensin and cathelicidin. On the contrary, SP strongly increases the mucin production and H_2O_2 release. While inoculated together, a lower apical PA growth is observed (- $3E^{+3}$ CFU/cm^2) suggesting an inhibition due to the presence of SP.

Conclusion

These results suggest that *in vitro* human airway epithelia is a useful model to study bacterial interaction on the human nasal mucosa.