Evaluation of in vitro 3D human nasal epithelium (MucilAir™) to study the mechanism of action of influenza A and B viruses

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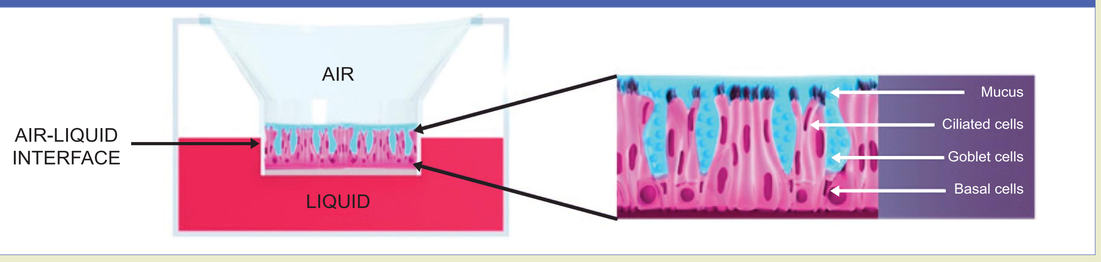
INTRODUCTION

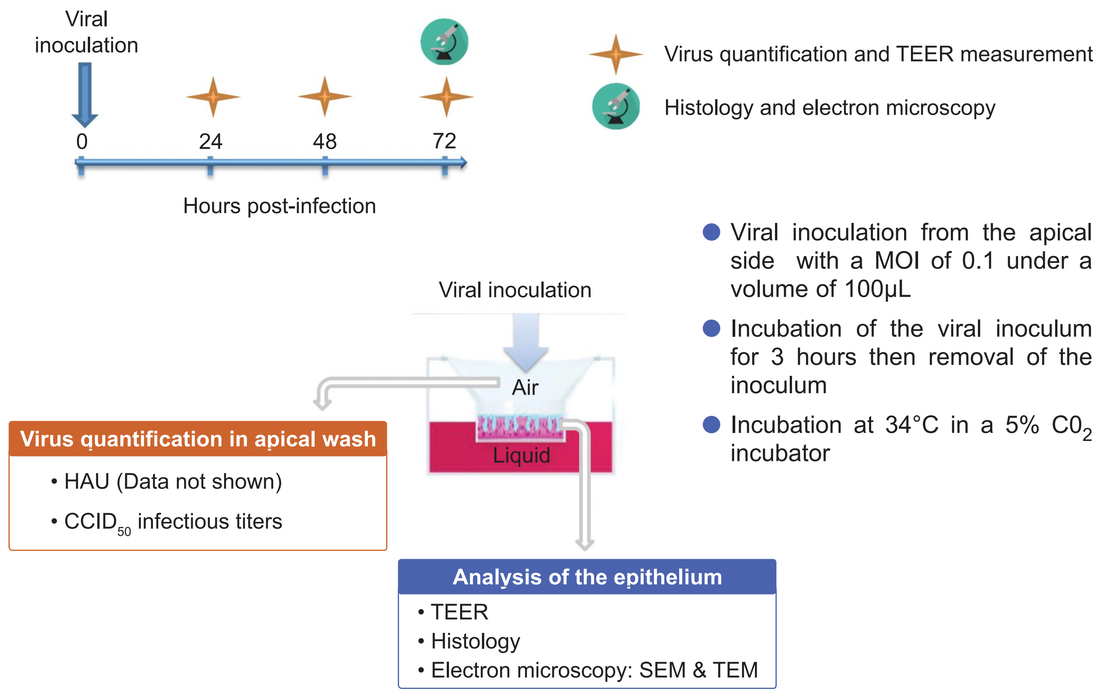
- Influenza virus causes disease by infecting nasopharynx mucosa. Therefore, appropriate *in vitro* models are useful to evaluate the mechanism of viral infection and the efficacy of new therapies or vaccines.
- In this study, we used commercially available, *in vitro* reconstituted 3D human upper airway epithelia (MucilAir™, Epithelix), fully differentiated and containing goblet, basal and ciliated cells showing cilia beating. This system is constituted of primary human epithelia cells freshly isolated from nasal biopsies seeded onto a semi-porous membrane.
- This human standardized nasal epithelium displays specific defense mechanisms comparable to the *in vivo* situation, such as mucus production, mucociliary clearance, and secretion of defensive molecules.

Schematic representation of MucilAir™



- Using this 3D model, we compared the replication of four influenza strains present in the 2016-2017 influenza vaccine:
  - A/PR8 reassortant strains: A/California/07/2009 (H1N1) X-179A and A/Hong Kong/4801/2014 (H3N2) X-263B
  - B strains: B/Brisbane/60/2008 and B/Phuket/3073/2013

METHODS



Virus quantification in apical wash

- HAU (Data not shown)
- $CCID_{50}$ infectious titers

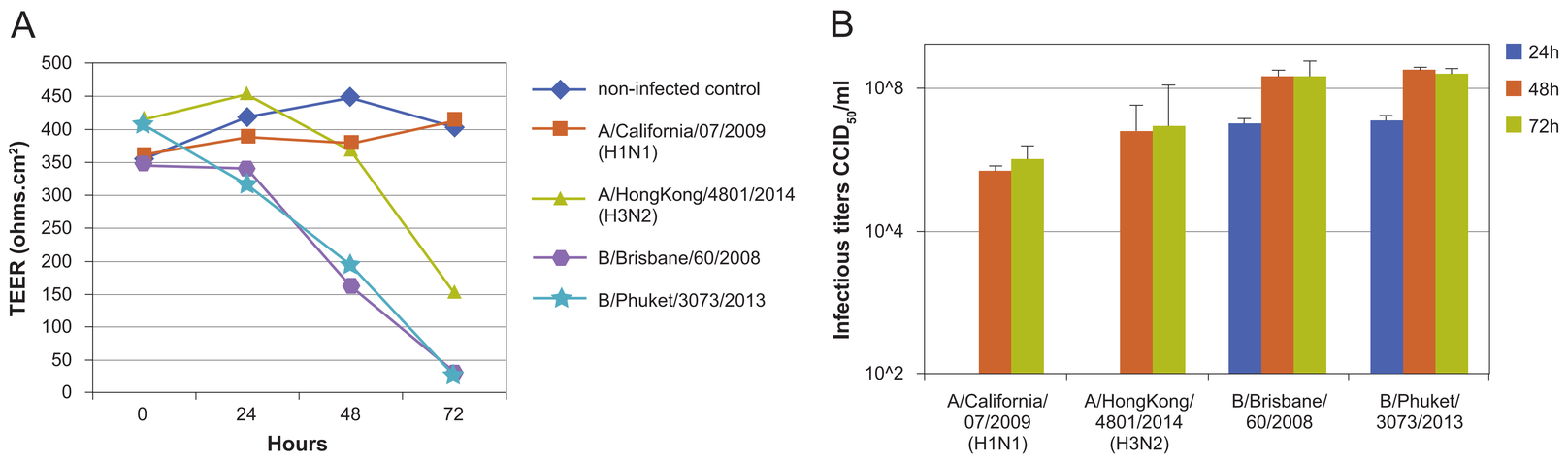
- Viral inoculation from the apical side with a MOI of 0.1 under a volume of 100µL
- Incubation of the viral inoculum for 3 hours then removal of the inoculum
- Incubation at 34°C in a 5% $C0_2$ incubator

Analysis of the epithelium

- TEER
- Histology
- Electron microscopy: SEM & TEM

RESULTS

COMPARISON OF THE KINETICS OF FOUR STRAINS PRESENT IN THE 2016-2017 INFLUENZA VACCINE



Mean and standard deviation for n=3 independent experiments

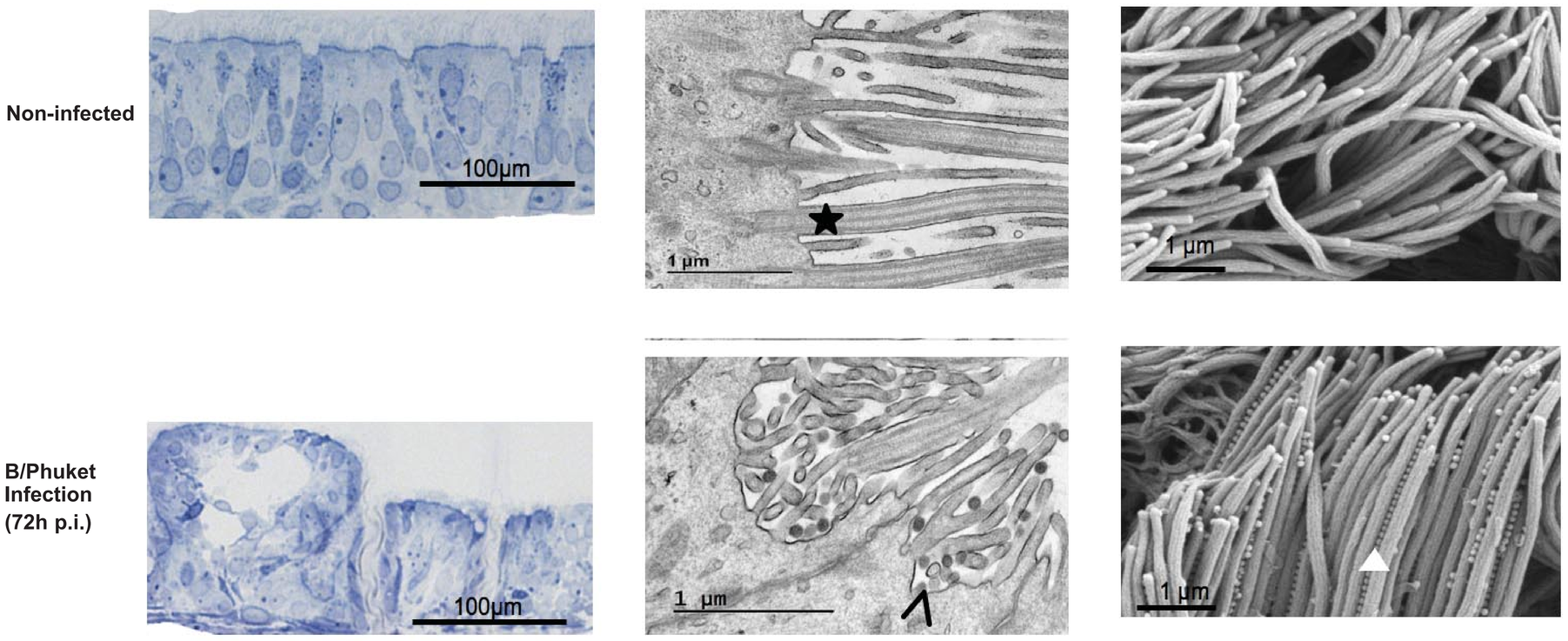
(A) TEER measurements

- During infection with the A/H3N2 strain, the TEER decreased while it remained the same for the A/H1N1 strain.
- For both B strains, a huge decrease of the TEER was measured until 72h p.i.

(B) $CCID_{50}$ infectious titers from the apical wash

- For the A strains, infectious titers were negative 24h post infection but became positive at 48h and increased until 72h to reach 10^6 to 10^7 $CCID_{50}$ /mL.
- For the B strains, positive titers were obtained as soon as 24h p.i. and plateaued between 48h and 72h reaching 10^8-10^9 $CCID_{50}$ /mL.

DAMAGE INDUCED BY THE INFLUENZA B/PHUKET/3073/2013 VIRUS ON THE NASAL MUCILAIR™ MODEL: MORPHOLOGICAL OBSERVATIONS



(A) Histology observation of a semi-thin section of the epithelium after toluidine blue staining. The damage of the epithelium is obvious 72h after infection with the B/Phuket strain.

(B) TEM observation of a thin section containing ciliated cells (black star: section of a cilia). After infection, the B virus buds from the ciliated cells (black arrow).

(C) SEM observation of the apical surface of the epithelium decorated with the ciliated cells. Interestingly, viruses are observed attached all along the cilia in single file (white arrow).

CONCLUSIONS

- **In this study we showed that the 3D human upper airway epithelia (MucilAir™) from Epithelix can support a productive A and B infection with the A strain-infection starting one day after the B strain-infection. Of note, infectious titers were also higher for the B strains compared to the A strains.**
- **B/Phuket infection caused a severe damage of the epithelium, as observed by histology. Epithelium damage was also evidenced by the dramatic decrease in TEER after infection with this strain. Electron microscopy confirmed the presence of virus budding from the ciliated cells, with viral particles in a single file all along the cilia.**
- **This 3D model, mimicking the in vivo upper airway epithelium infected by influenza strains, will be used to study and compare the mechanism of action of different strains of influenza viruses.**

Cell Culture Infectious Dose 50 % ($CCID_{50}$)
Hemagglutination Unit (HAU)
Multiplicity Of Infection (MOI)
Post-Infection (p.i.)
Scanning Electron Microscopy (SEM)
Transmission Electron Microscopy (TEM)
Trans-Epithelial Electrical Resistance (TEER)

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