Comparison of respiratory virus infection between human nasal epithelial cell monolayer and air-liquid interface 3D culture
Kazuhiro Ito¹, Song Huang² and Samuel Constant²,
¹RespiVert Ltd, London, United Kingdom and ²Epithelix Sàrl, Geneva, Switzerland

Introduction
Viral infection has been implicated in exacerbations of COPD and Asthma¹, and Human Rhinovirus (HRV) is often detected in patients during exacerbations². Therefore, appropriate in vitro model is required to evaluate infectious mechanism and the efficacies of new therapy. In this study, we compared the replication of virus, such as human rhinovirus (HRV16), respiratory syncytial virus (RSV A2) and influenza virus (WSN33, H1N1) in 3D culture air-liquid interface human nasal epithelial cells (NEC-3D) and NEC monolayer culture (NEC-M).

Methods

Virus
HRV16 and RSV-A2 were obtained from ATCC and WSN33, H1N1 was obtained from HPA, UK.

Cells
Nasal epithelial cells (NEC) were purchased from Epithelix Sàrl and cultured as monolayer. Air-liquid interface cultured nasal epithelial cells were also obtained from Epithelix Sàrl (MucilAir™).

Infection
Before-infection, cells were washed with media to remove mucus. HRV16 (approx. 1 MOI, 5.9 x 10⁻⁶ TCID₅₀), RSV A2 (approx. 0.1 MOI, 5.7 x 10⁻⁶ TCID₅₀) and WSN33 (approx. 1 MOI, 5.7 x 10⁻⁶ TCID₅₀) were infected to ALI cultured insert. HRV16 (approx. 1 MOI, 2.8 x 10⁻⁶ TCID₅₀), RSV A2 (approx. 0.1 MOI, 2.4 x 10⁻⁶ TCID₅₀) and WSN33 (approx. 1 MOI, 2.8 x 10⁻⁶ TCID₅₀) were infected to nasal epithelial monolayer culture (96 well plate). The cells were washed out with PBS after 1hr virus absorption. The cells were then stained with Crystal Violet for confirmation. The amount of virus required to infect 50% of cells (TCID₅₀) was 3 at 5 days post-infection, and peaked at 6 days (6.3 TCID₅₀) (Figure 1). RSV A2 replicated well in both cells with peak TCID₅₀ of 4 in monolayer and 5.3 in 3D-NEC (Figure 2). Influenza WSN33 only weakly replicated in NEC monolayer with peak TCID₅₀ of 2, but it replicated well in 3D-NEC and the peak TCID₅₀ was 6.8 at 5 days post-infection (Figure 3). All virus induced IL-8 production and the induction level was less than 10 fold compared with basal IL-8 level in NEC monolayer, but it was induced by more than 400 fold in 3D-NEC (Figure 4).

Conclusions
All respiratory virus used in this study, particularly HRV16 and H1N1, replicated more in NEC-ALI 3D than NEC-Monolayer. IL-8 production was also higher in NEC-ALI 3D than NEC-Monolayer. This unique and robust 3D culture cell system (MucilAir™) will be a better in vitro model for evaluation of respiratory virus infection.

Bibliography
2) Leung TF, To MY, Yeung AC, Wong YS, Wong GW, Chan PK. Chest. 2009 Sep 11.

Figure 1
HRV16 replicated in NEC monolayer and the TCID₅₀ was 3 at 5 days post-infection. In contrast HRV16 replicated better in 3D-NEC and TCID₅₀ reached to 4.5 at 4 days post-infection, and peaked at 6 days (6.3 TCID₅₀) (Figure 1). RSV A2 replicated well in both cells with peak TCID₅₀ of 4 in monolayer and 5.3 in 3D-NEC (Figure 2). Influenza WSN33 only weakly replicated in NEC monolayer with peak TCID₅₀ of 2, but it replicated well in 3D-NEC and the peak TCID₅₀ was 6.8 at 5 days post-infection (Figure 3). All virus induced IL-8 production and the induction level was less than 10 fold compared with basal IL-8 level in NEC monolayer, but it was induced by more than 400 fold in 3D-NEC (Figure 4).

Figure 2

Figure 3

Figure 4

Figure 1