Epithelix

in vitro Solutions for Respiratory Diseases and Chemical Testing

Efficient replication of respiratory syncytial virus induces a decrease of mucociliary clearance in human small airway 3D culture

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Introduction

Respiratory syncytial virus (RSV) infection causes upper and lower respiratory tract infections and is the most common cause of bronchiolitis and pneumonia in young children. To understand RSV pathogenesis in humans and to test new molecules for alleviating the diseases, an *in vitro* RSV infection platform based on three dimensional (3D) fully differentiated human airway epithelia cultured at air-liquid interface was developed.

One such model, SmallAir™, representing the small airway epithelia with characteristic cell types (basal, ciliated and Club cells) was successfully infected with clinical isolate of RSV B.

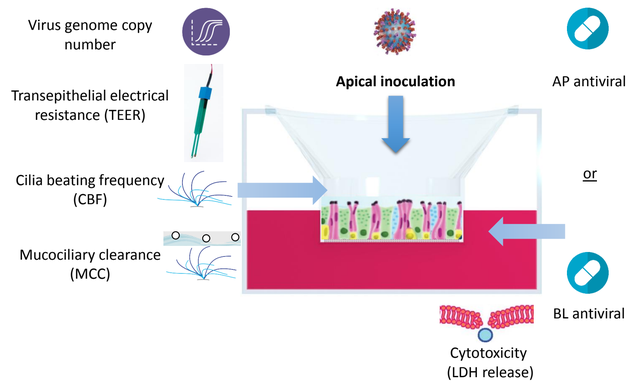
Materials and Methods

SmallAir™

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

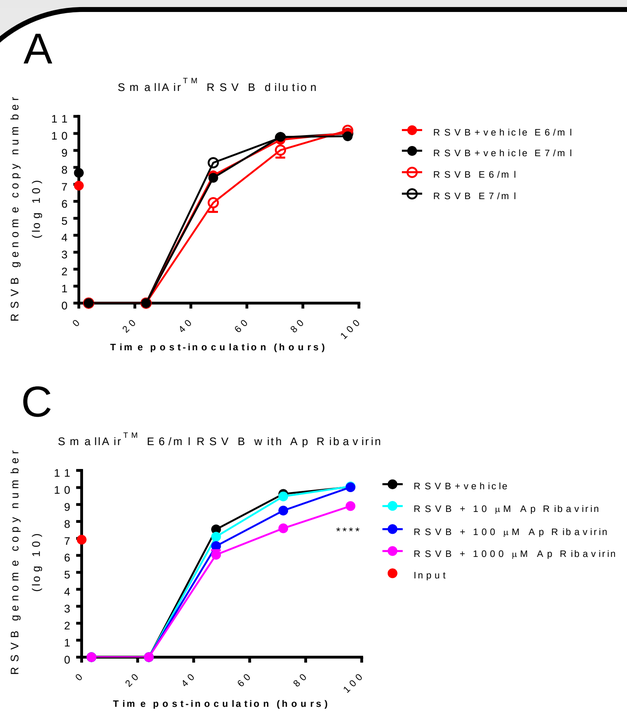
Transversal cross section of SmallAir™ with alcian blue and hematoxylin-eosin staining (top) and Club cell specific CC-10 immunohistochemistry (below). Scale bar: 40 μm.

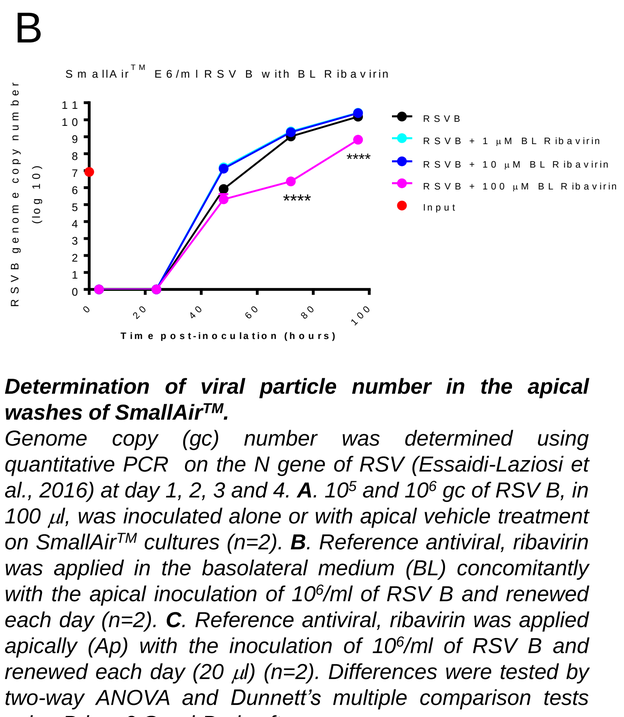
Testing strategy



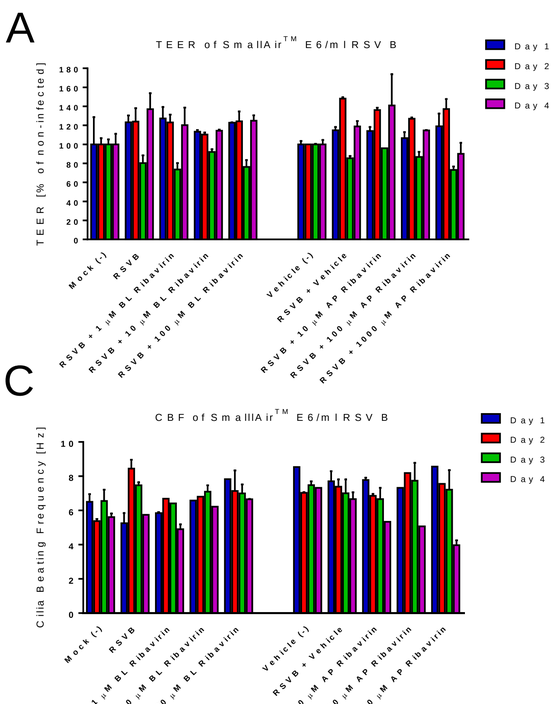
Clinical isolate of Respiratory Syntitial Virus (RSV) B was inoculated at 10^5 and 10^6 genome copy in 100 μl on the apical side of SmallAir™ (SA069301) for 3 hours. After an apical wash, virus genome copy number was determined at 1, 2, 3 and 4 days post-inoculation on the apical side of the cultures. Reference antiviral, ribavirin, was applied concomitantly with the virus either apically in 20 μl (Ap; 10, 100, 1000 μM) or basally (BL; 1, 10, 100 μM). Different functional end points were assessed at day 1, 2, 3 and 4 from both sides of cultures.

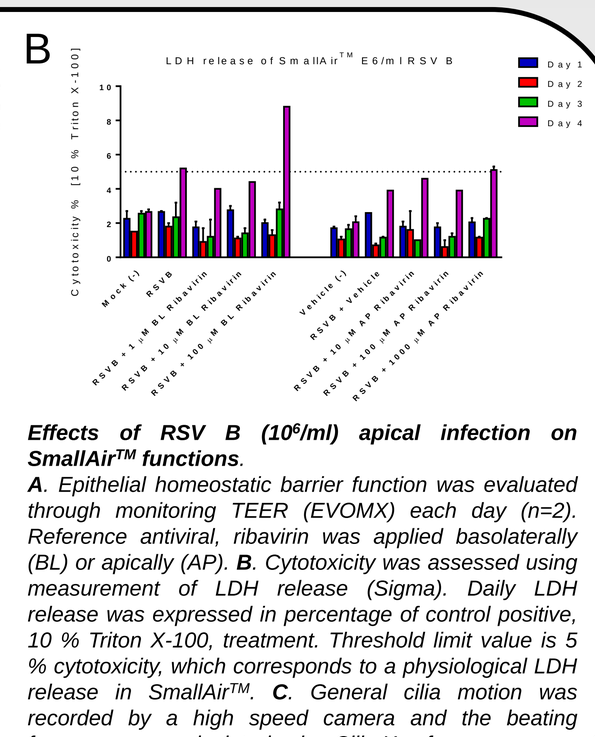
Results



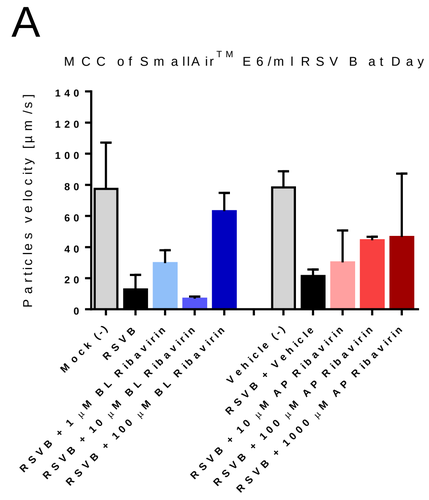


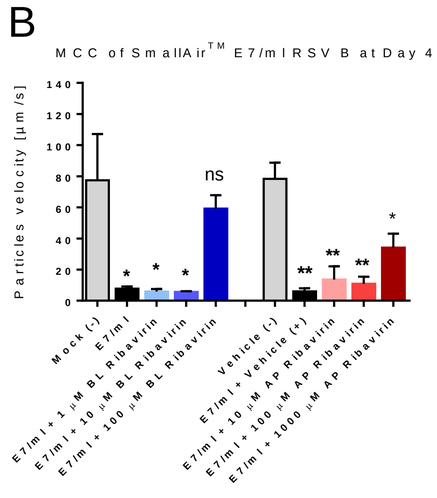
Determination of viral particle number in the apical washes of SmallAir™.
*Genome copy (gc) number was determined using quantitative PCR on the N gene of RSV (Essaidi-Laziosi et al., 2016) at day 1, 2, 3 and 4. **A**. 10^5 and 10^6 gc of RSV B, in 100 μl, was inoculated alone or with apical vehicle treatment on SmallAir™ cultures (n=2). **B**. Reference antiviral, ribavirin was applied in the basolateral medium (BL) concomitantly with the apical inoculation of 10^6/ml of RSV B and renewed each day (n=2). **C**. Reference antiviral, ribavirin was applied apically (Ap) with the inoculation of 10^6/ml of RSV B and renewed each day (20 μl) (n=2). Differences were tested by two-way ANOVA and Dunnett's multiple comparison tests using Prism 6 GraphPad software.*





Effects of RSV B (10^6/ml) apical infection on SmallAir™ functions.
***A**. Epithelial homeostatic barrier function was evaluated through monitoring TEER (EVOMX) each day (n=2). Reference antiviral, ribavirin was applied basolaterally (BL) or apically (AP). **B**. Cytotoxicity was assessed using measurement of LDH release (Sigma). Daily LDH release was expressed in percentage of control positive, 10 % Triton X-100, treatment. Threshold limit value is 5 % cytotoxicity, which corresponds to a physiological LDH release in SmallAir™. **C**. General cilia motion was recorded by a high speed camera and the beating frequency was calculated using Cilia X software.*





Effects of RSV B apical infection on the mucociliary clearance in SmallAir™.
*Mucociliary clearance was evaluated through microscopic monitoring of polystyrene microbeads (Sigma) applied on the apical surface. Particle velocity was quantified from the recorded video using Image Pro Plus software. Differences were tested by one-way ANOVA using Prism 6 GraphPad software and Dunnett's multiple comparison tests were used to compare every mean to control mean. **A**. RSV B was inoculated at 10^6/ml **B**. 10^7/ml concentration. Reference antiviral, ribavirin was applied basolaterally (BL) or apically (AP) (n=2 and 2).*

Summary

- With an initial inoculum of 10^5 or 10^6 RSV B viruses, the genome copy number reached 10^{10} gc/ml 4 days post-inoculation in the apical washes.
- The RSV B infection did not impair the tissue integrity nor cause cytotoxicity.
- Solely the mucociliary clearance showed a dramatic decrease at 4 days post-inoculation of RSV B (22 and 9 % of the non-infected control for 10^6 and 10^7/ml virus inoculation, respectively).
- Ribavirin, as reference antiviral against RSV, applied either apically (1-10-100 μM) or basally (10-100-1000 μM), inhibited viral replication in a dose-dependent manner and partially prevented the decrease of the mucociliary clearance.

Conclusion

These results demonstrate that SmallAir™ is a pertinent tool to perform anti-RSV drug screening via airborne or systemic delivery.