

To pool or not to pool cells: A retrospective analysis of human airway epithelia reconstituted in vitro

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Human airway epithelial cultures cultured at the air liquid interface (ALI) in Transwell as became a gold standard through years. Due to their specific genetic background, the test results based on single donor may cause biased outcome. To overcome these problems, MucilAir™-Nasal-Pool were reconstituted with a mixture of primary human nasal epithelial cells from 14 different healthy donors with similar characteristics. 8 generations of MucilAir™-Nasal-Pool (P6 to P13) were produced over 13 years (since 2012), each pool being reconstituted with different donors.

The accumulated data demonstrated that the quality of the epithelia was excellent in terms of steady state and stimulated parameters, such as TEER, Cilia Beating Frequency (CBF), mucociliary clearance (MCC), cytokine secretion (IL-8) release. Furthermore, all the epithelia could be infected by various respiratory virus, such as Influenza (H1N1). 14 days repeated dose toxicity of Formaldehyde was tested on pools from 3 anatomical regions: nasal, bronchial and small-airways (MucilAir™-Nasal-Pool vs MucilAir™-Bronchial-Pool vs SmallAir™-Pool).

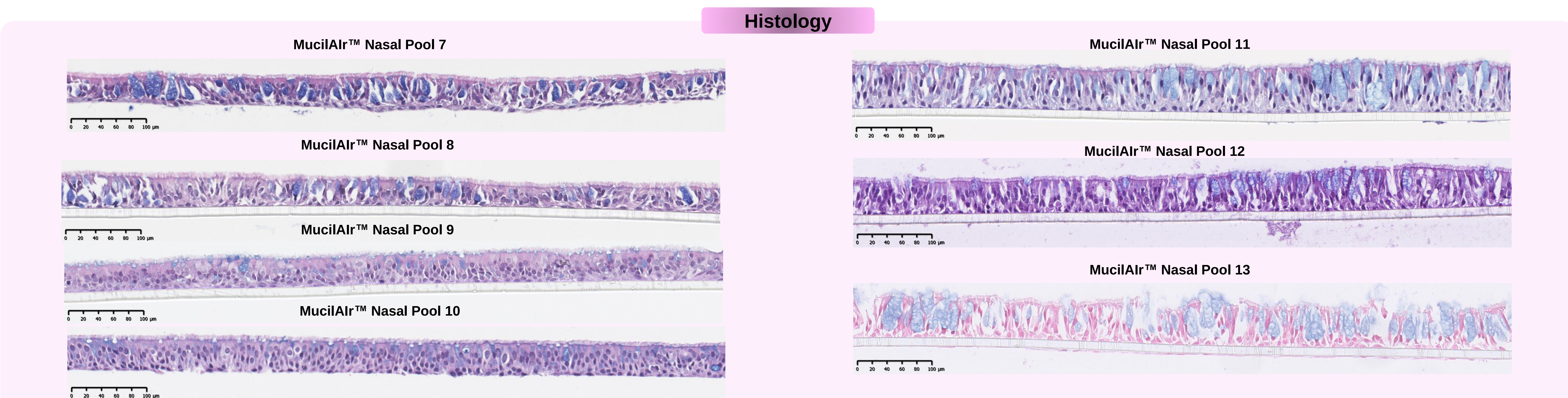


Figure 1. Cross sections of differentiated MucilAir™ Nasal Pool, 5 weeks after Air Liquid Interface (ALI)

TEER measurement for tissue integrity (EVOM™)

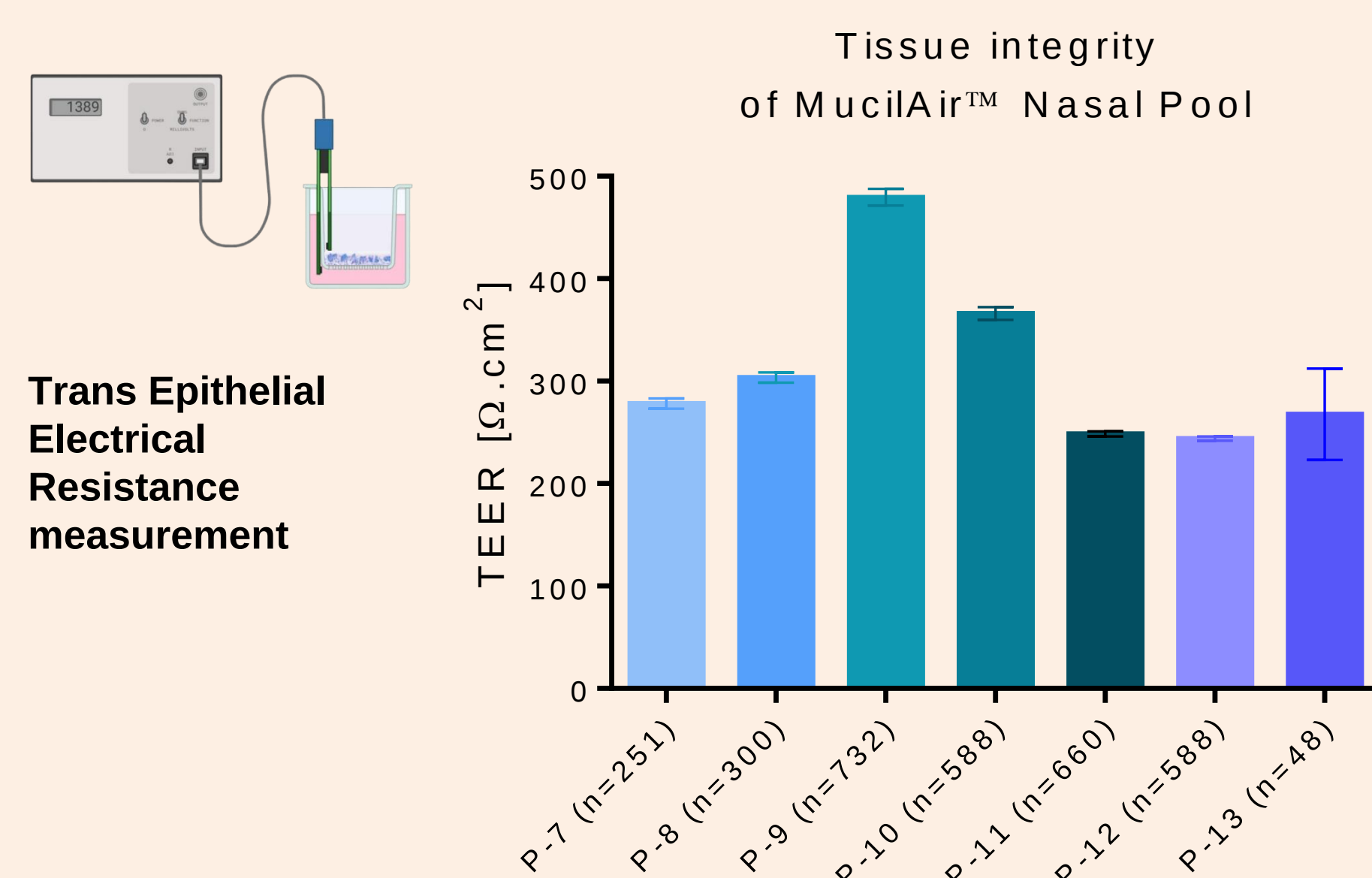


Figure 2. Transepithelial electrical resistance (TEER) measurement. TEER MucilAir™ Nasal Pool were assessed around 5 weeks post-ALI. TEER reflects the state of the epithelium barrier function, which is typically between 200 and 600Ω.cm². TEER values remain consistent across different pool generations.

Inflammatory response to cytomix stimulation

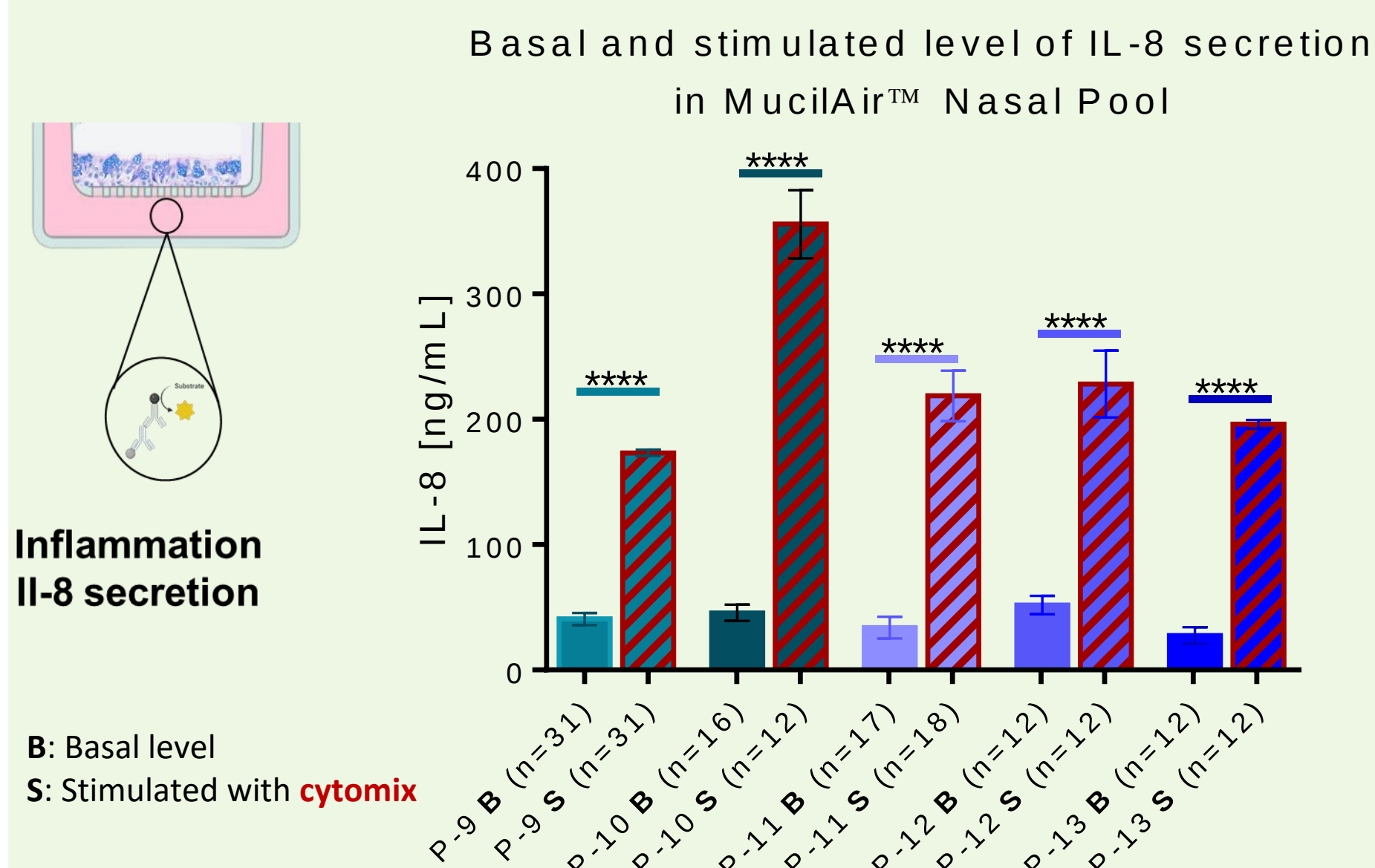


Figure 3. Cytomix induced stimulation of IL-8 secretion across several MucilAir™-Nasal-Pool generations (Student's t test, Prism 6.0 GraphPad, ****p<0.0001). The gap between basal and stimulated levels (averaging 6 times higher) enabled the different MucilAir™-Nasal-Pools to study the inflammatory effects of compounds.

Cilia beating frequency

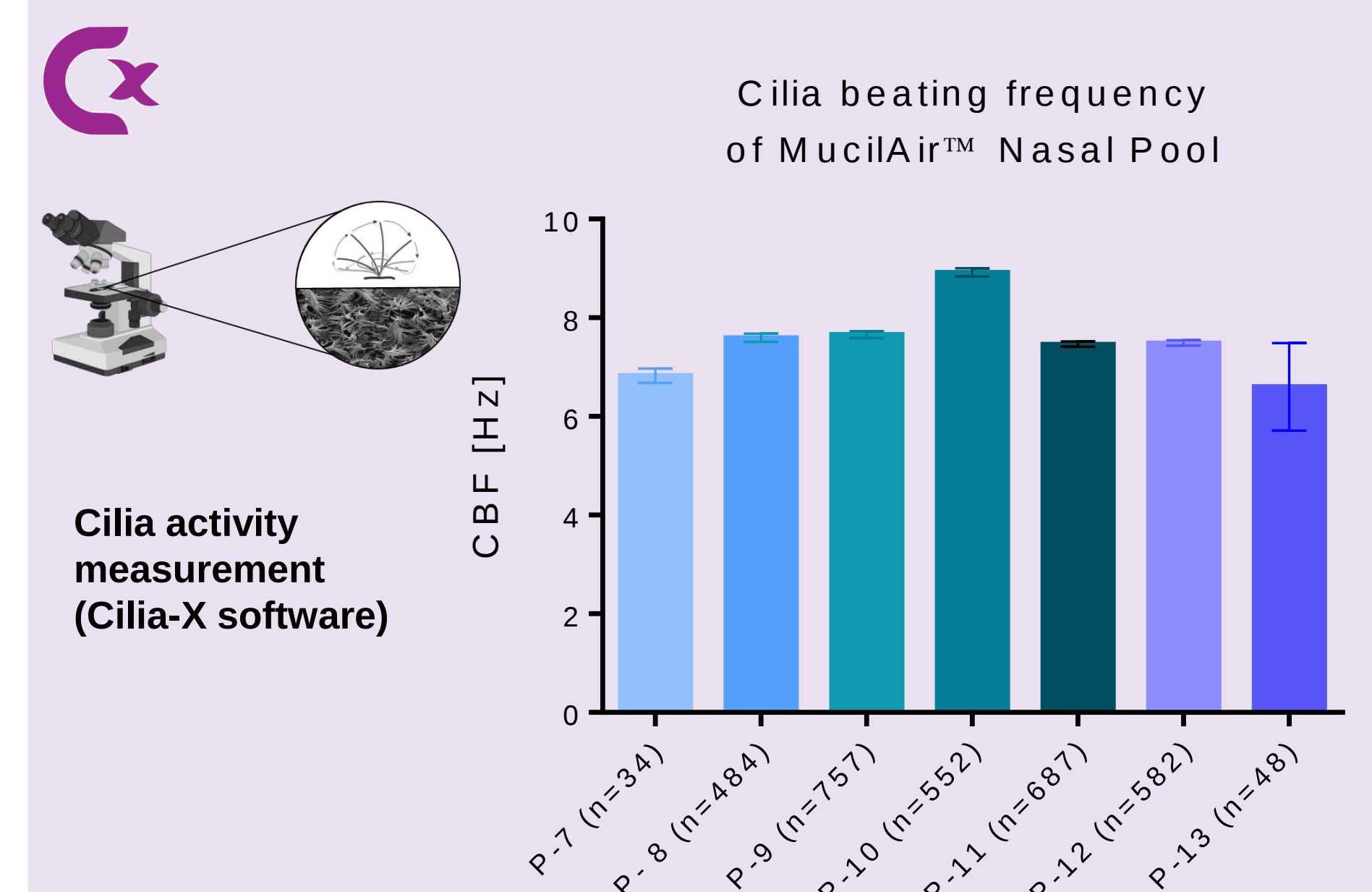


Figure 4. Similar cilia beating frequency (CBF) were obtained across several MucilAir™-Nasal-Pool generations (Cilia-X software). All produced pools allowed the study of compounds' effects on cilia activity.

Mucociliary clearance

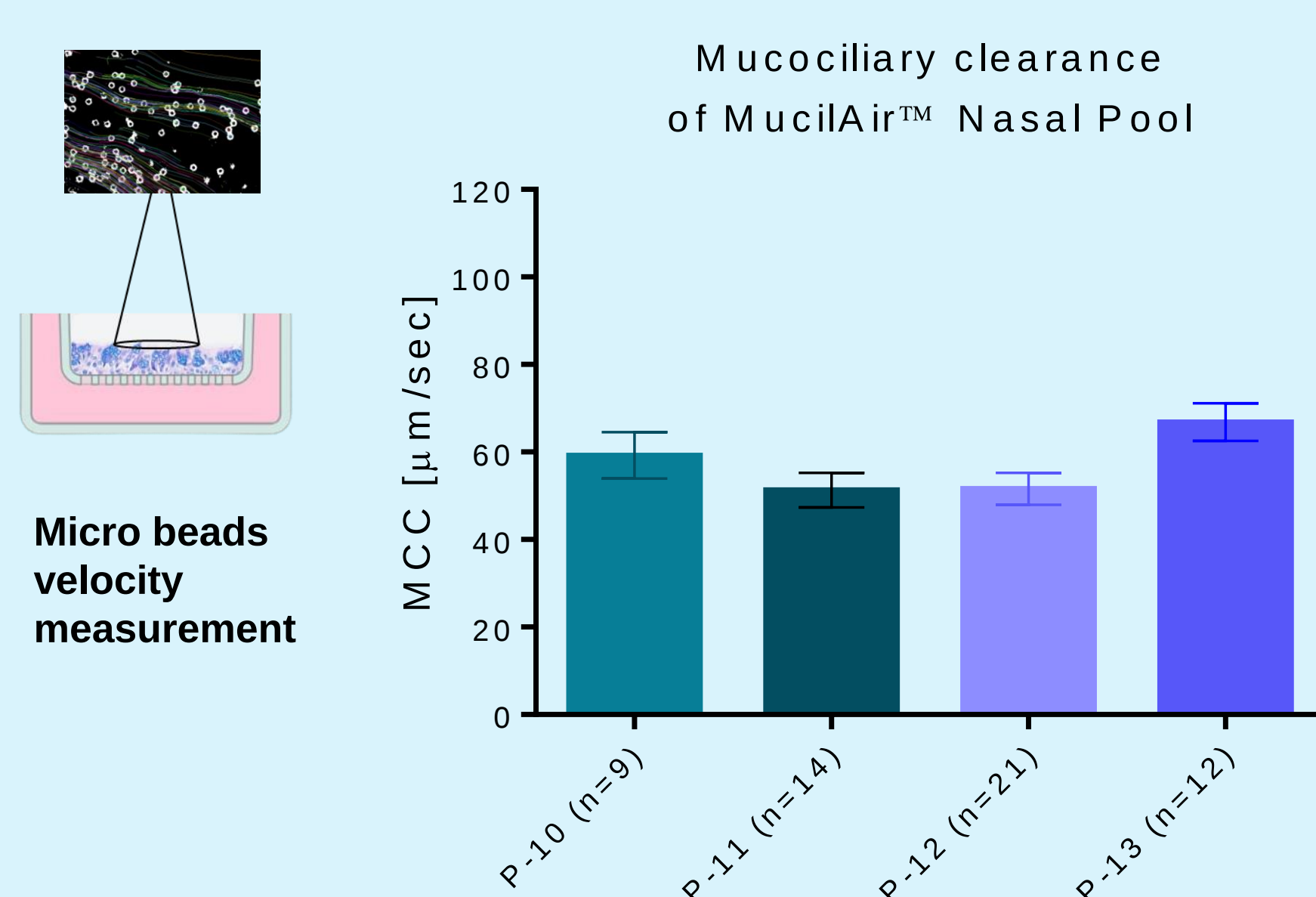


Figure 5. Mucociliary Clearance (MCC) is a respiratory tract purification process. MCC measurement reflects the ability of respiratory epithelium to trap and evacuate particles. Similar micro beads velocities were obtained across several MucilAir™-Nasal-Pool generations (around 50 μm/sec).

Viral infection

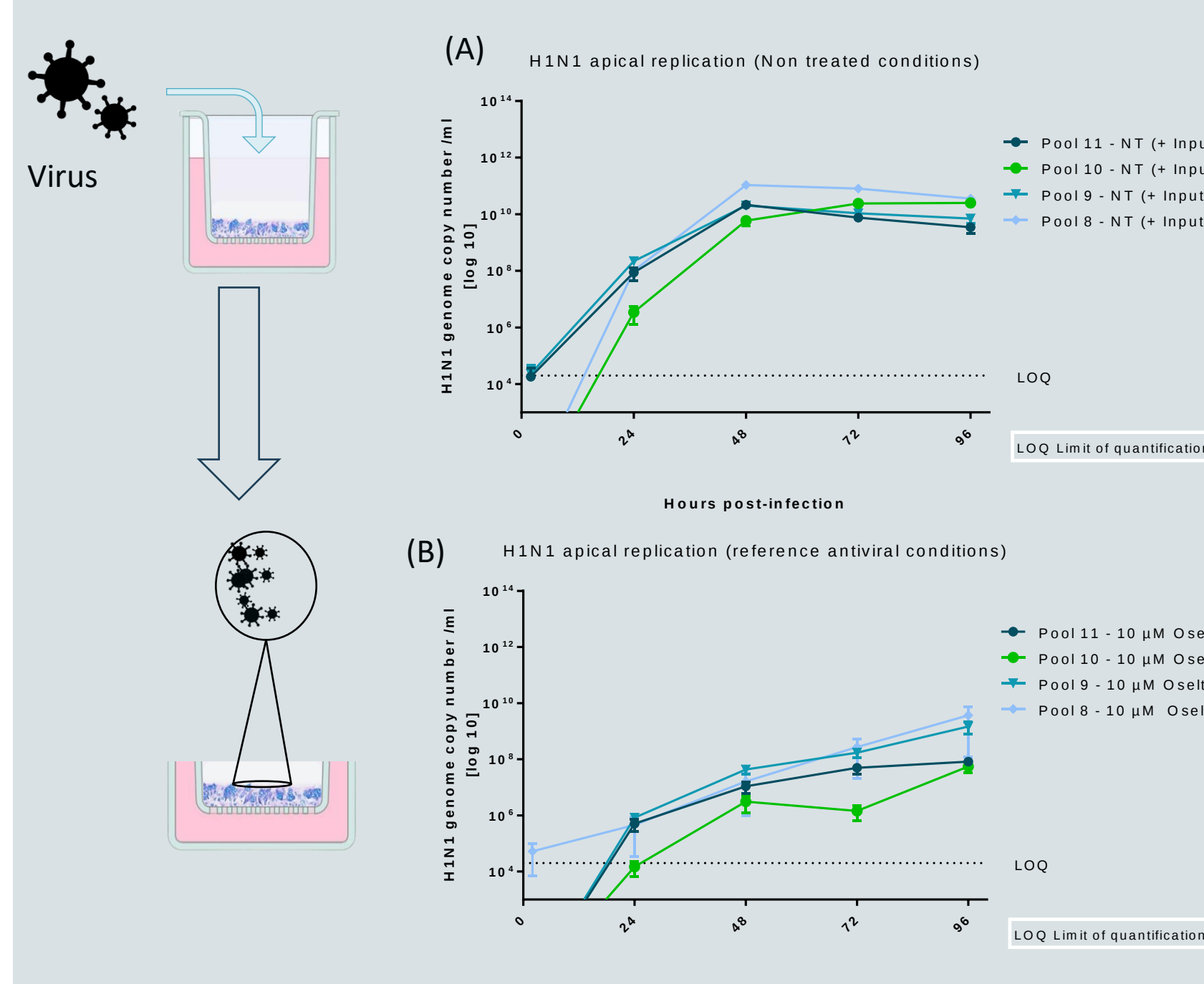


Figure 6. Influenza A (H1N1) infection using MucilAir™ Nasal Pool in infected non treated condition (A) and in infected condition with a reference antiviral: Oseltamivir (B).

Repeated exposure to Formaldehyde

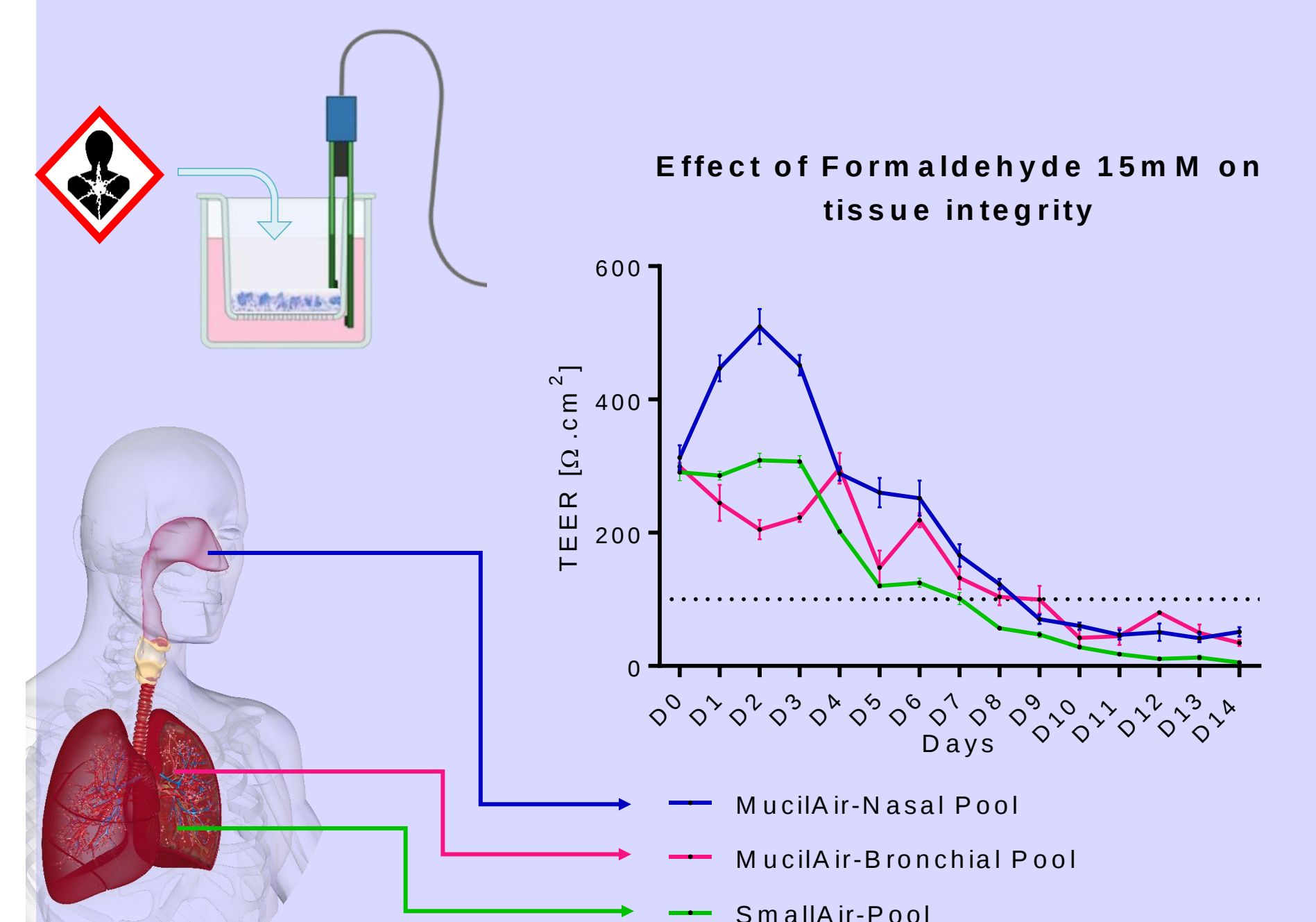


Figure 7. MucilAir™-Nasal-Pool, MucilAir™-Bronchial-Pool and SmallAir™-Pool are exposed for 14 days to 15mM of Formaldehyde (10 μl, apical). Tissue integrity is evaluated through TEER measurement for toxicity evaluation.

CONCLUSION

Data suggest that pooled versions of ALI maintain similar morphology and functions across generations and over a span of 13 years. This strategy allows accomplishing large research projects requiring substantial amount of ALI cultures. Moreover, the responses of the pooled version could avoid potential biases of single donor version. On the other hand, for modelling specific respiratory diseases, or searching for disease-causing genes, mono-donor ALI could be more relevant.