The major respiratory diseases such as asthma and COPD are inflammatory in nature. By expressing a large panel of cytokines and chemokines, the airway epithelia plays an essential and central role in these inflammatory responses. Therefore, the airway epithelium is an ideal target of anti-inflammatory drugs. Based on a long shelf-life 3D in vitro cell model of the human airway epithelium (MucilAir), which is fully differentiated and closely mimicking the morphology and function of the native tissues, we tested two classes of reference anti-inflammatory drugs: the steroids (Budesonide, Fluticasone and Mometasone) and the non-steroidal anti-inflammatory drug (NSAID) (Piroxicam and Ibuprofen). In order to simulate the chronic inflammation, the epithelia were treated with TNF-α, or LPS, or 10% FBS every day for 5 days. The anti-inflammatory drugs were also added into the culture medium at three different concentrations: 10, 100 and 200 μM. IL-8 released by the airway epithelial cells was used as an endpoint for assessing the efficacy of the drugs. These results were obtained from epithelia reconstituted with cells from single donor, but also confirmed on epithelia made of cells from a pool of 14 donors, excluding the donor-donor variations.

The advantages of MucilAir™

- It is composed of primary human respiratory cells.
- It mimics the morphology and functions of the native human airway epithelium.
- It has a unique shelf-life of 12 months.
- Epithelia from different pathologies are available (asthma, COPD, CF, allergic rhinitis).
- It is ready and easy to use.

### TNF-α treatment

![Graph showing Anti-inflammatory effect of 5 molecules at 10µM on IL-8 secretion](image)

### LPS treatment

![Graph showing Anti-inflammatory effect of 5 molecules at 10µM on IL-8 secretion](image)

### FBS treatment

![Graph showing Anti-inflammatory effect of 5 molecules at 10µM on IL-8 secretion](image)

### Discussion

As reported, the steroids significantly reduced the IL-8 release at all three experimental settings, but Fluticasone and Mometasone seem to be much more potent than Budesonide: Fluticasone and Mometasone showed 4 fold inhibition already at 10 µM, while to achieve such level of inhibition, 100 µM Budesonide was needed. As for NSAID, the results were less clear-cut: At 10 µM, Piroxicam seems to potentiate the pro-inflammatory effects of TNF-α and 10% FBS: the amount of IL-8 was almost doubled when compared with the TNF-α or 10% FBS alone. However, an inhibitory effect of Piroxicam was observed at higher concentrations (100 and 200 µM). Surprisingly, Piroxicam seems to be able to block the inflammatory effect of LPS at later time points (from D2) and at higher concentrations.

Ibuprofen showed similar inhibitory profile as Piroxicam but it didn't show synergistic effects with TNF-α, LPS nor 10% FBS on IL-8 release. The effects of these drugs are not correlated with the toxicological profiles. But due to the relatively high effective doses, the observed effects on IL-8 release might be off-target effect which may explain why NSAIDs are not widely used for treating respiratory diseases like asthma.

### Conclusions

1. MucilAir™ is a reliable and convenient tool for screening, validating and ranking the anti-inflammatory drug candidates potency, especially for the steroids
2. Standard Operating procedures are accessible.