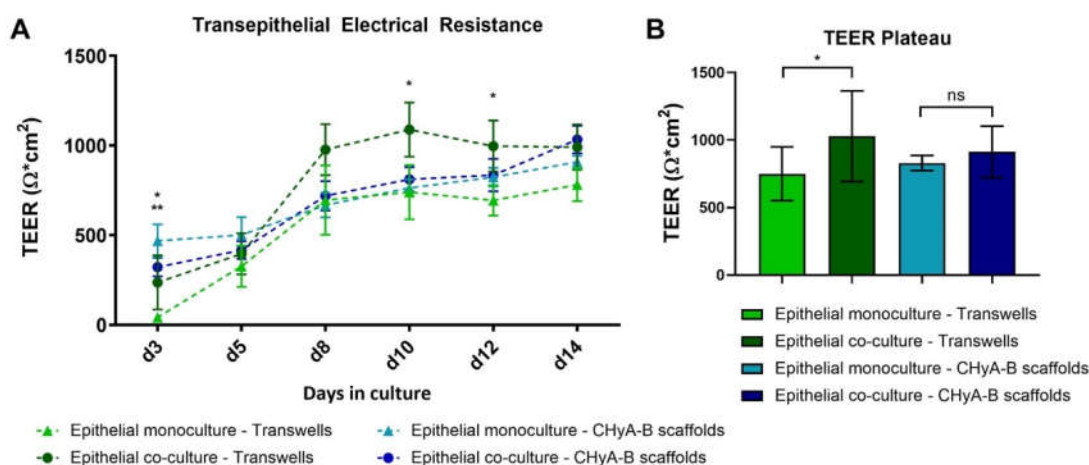


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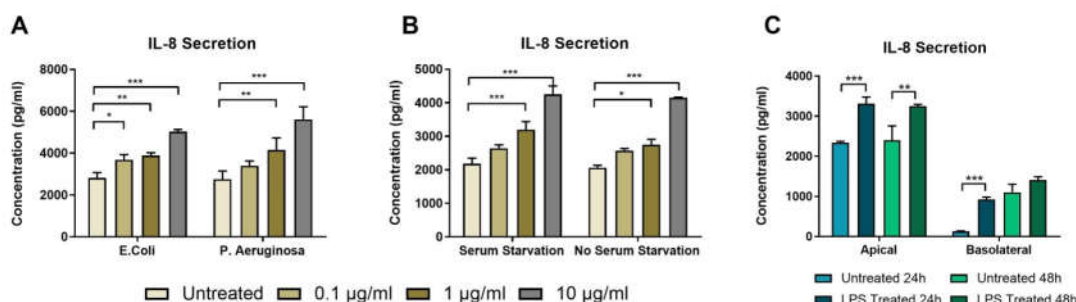
# A Tissue-Engineered Tracheobronchial In Vitro Co-Culture Model for Determining Epithelial Toxicological and Inflammatory Responses

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## Supplementary materials

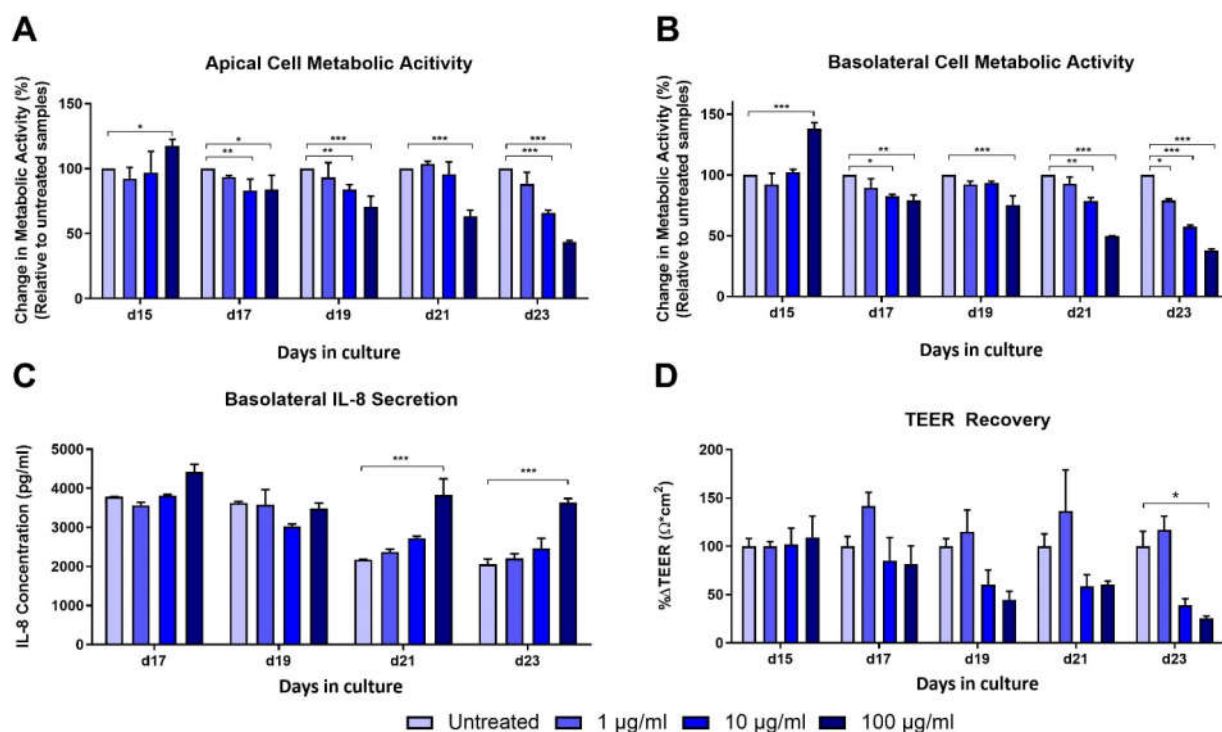


**Figure S1:** Barrier integrity from epithelial monocultures and co-cultures on Transwell® inserts and CHyA-B scaffolds. (A) TEER values measured at day 3, 5, 8, 10, 12, and 14 from Calu-3 epithelial monocultures (light green) and Calu-3 Wi38 epithelial co-cultures (dark green) on Transwell® inserts; and Calu-3 epithelial monocultures (light blue) and Calu-3 Wi38 epithelial co-cultures on CHyA-B scaffolds under ALI conditions. (B) Average TEER values of epithelial cultures following plateau of electrical resistance measured at day 10, 12, and 14. Results displayed as mean  $\pm$  SEM ( $n = 7$ ; \* $p < 0.05$ , \*\* $p < 0.01$ )

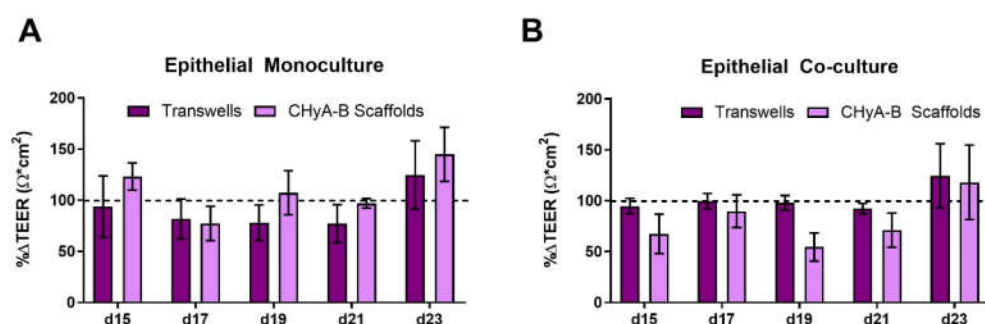


**Figure S2.** Optimisation of LPS dose response in Calu-3 epithelial cells. (A) IL-8 secretion from non-polarised Calu-3 epithelial cells following LPS exposure with 10, 1, and 0.1  $\mu\text{g/ml}$  of *E. coli* and *P. aeruginosa* derived LPS. (B) IL-8 secretion from non-polarised Calu-3 epithelial cells following LPS exposure 10, 1, and 0.1  $\mu\text{g/ml}$  of *P. aeruginosa* derived LPS in 1% (serum starvation) and 10% (no serum starvation) serum Calu-3 medium. (C) IL-8 secretion from polarised Calu-3 epithelial cells on Transwell® inserts

following 10 µg/mL of *P. aeruginosa* derived LPS for an exposure time of 24 and 48 h. Cytokine release was quantified in both apical and basolateral compartments after bacterial challenge. Results displayed as mean ± SEM ( $n = 1$  performed in quadruplicates; \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ ).



**Figure S3.** Optimisation of drug-mediated challenge in polarised Calu-3 epithelial cells on Transwell® inserts with 1, 10, and 100 µg/mL of bleomycin for a 24 h exposure time. (A,B) Apical and basolateral cell metabolic activity from polarised Calu-3 epithelial cells. (C) Basolateral IL-8 secretion from polarised Calu-3 epithelial cells. (D) TEER recovery from polarised Calu-3 epithelial cells on Transwell® inserts, TEER recovery values normalised to the recovery of untreated samples. Results displayed as mean ± SEM ( $n = 1$  performed in quadruplicates; \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ ).



**Figure S4:** TEER measurements following LPS exposure. TEER recovery of Calu-3 epithelial monocultures (A) and Calu3 Wi38 epithelial co-cultures (B) on Transwell® inserts and CHyA-B scaffolds following bacterial challenge with 10 µg/mL of *P. aeruginosa* derived LPS for 24 h. TEER recovery values normalised to the recovery of untreated cultures. Results displayed as mean ± SEM ( $n = 3$ ).

