Developing a model to test microplastic impact on lung epithelial barriers formation and functionality

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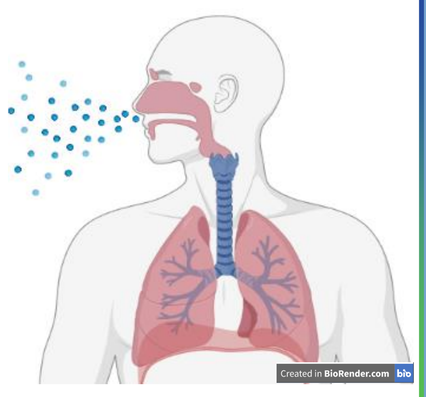
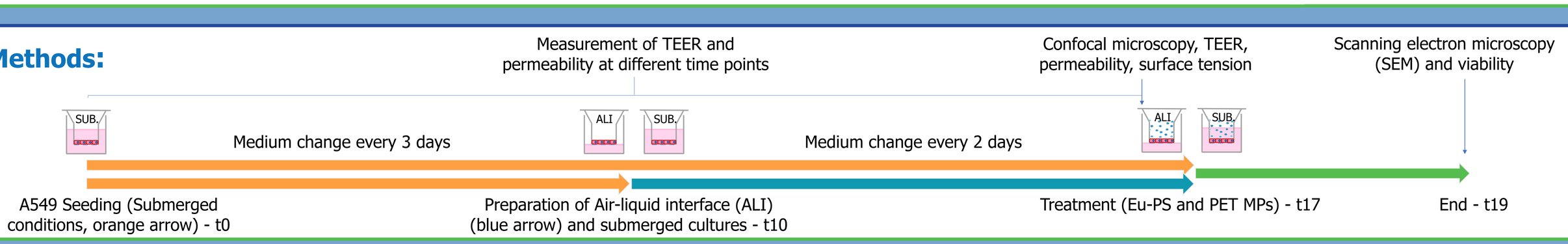
Background: Nano- and microplastics (NPs/MPs) are plastic particles smaller than 5 mm. They can be manufactured for a specific purpose or released into the environment through the degradation of plastic objects and NP/MP-containing products. One of the major routes of exposure for humans is via inhalation of indoor air. Studies have already reported that airborne particles can deposit or accumulate in the lungs causing occupational diseases in textile workers. The aim of this study is to develop and test a model for the evaluation of the impact of polyethylene terephthalate (PET; 1.12 ± 0.14 µm) and Eu-loaded polystyrene (PS; 300 nm) MPs on human health, with particular attention to the integrity and functionality of the lung barrier in terms of cell viability, surfactants and tight junctions production, barrier permeability, and trans-epithelial electrical resistance (TEER).



Methods:



Result 1 – Cells grown in ALI or Submerged conditions do not present differences in terms of permeability and TEER.

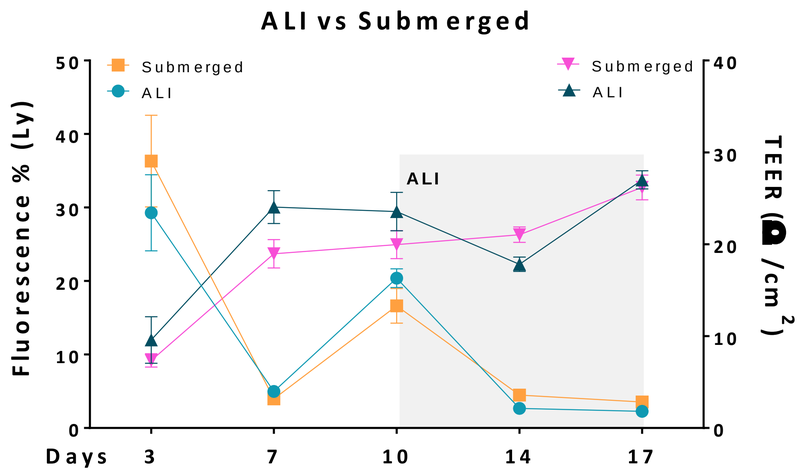


Figure 1: *overtime assessment of TEER and epithelial permeability of A549 cells grown on plastic inserts for 17 days either in submerged or ALI conditions. Values were obtained using EVOM3 and the lucifer yellow (Ly) assay.*

Result 2 – ALI grown cells produce more surfactants (lower tension) than cells grown in submerged conditions

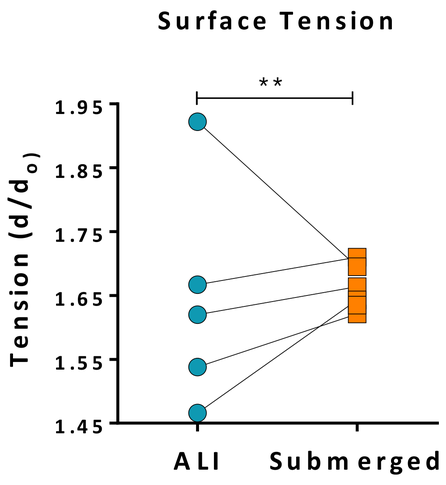
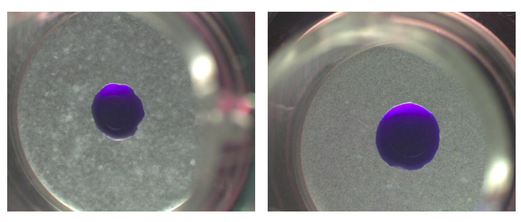


Figure 2: *Image showing the differences in surface tension of A549 grown either in ALI or submerged conditions. Values were calculated after 17 days of culture using the drop-spreading method (see below).*



Result 3 – Occludins are more expressed in ALI culture than in cells grown in submerged conditions

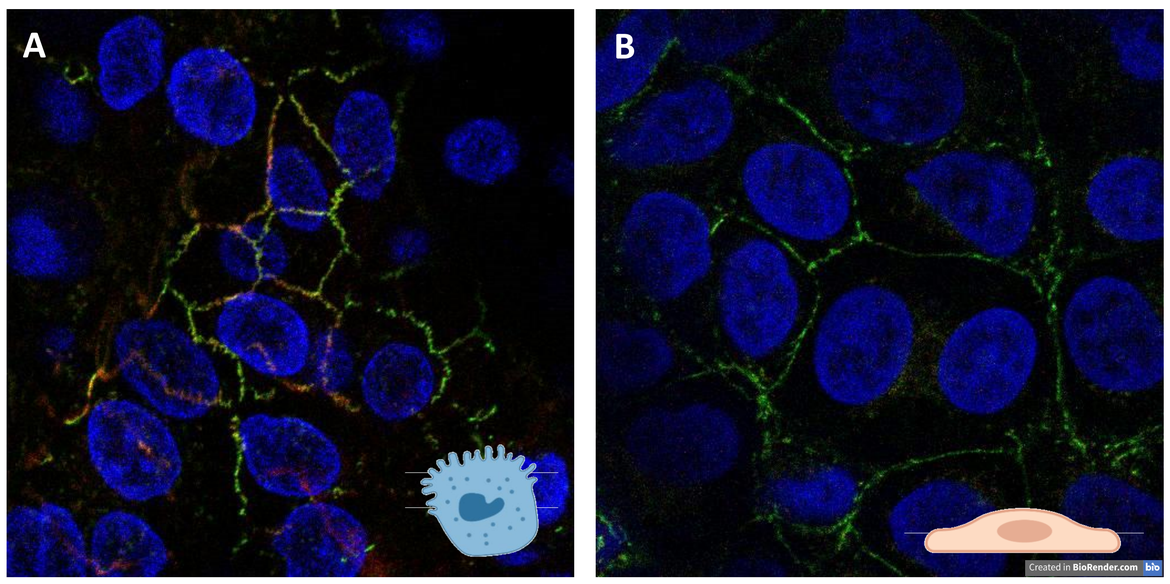


Figure 3: *Confocal microscopy of A549 cells grown for 17 days either in (A) ALI or (B) submerged conditions. Mag. 1000x.* ***nucleous****,* ***occludins****,* ***ZO-1.***

Result 4 – MPs may affect cell viability after 48h of treatment (preliminary results)

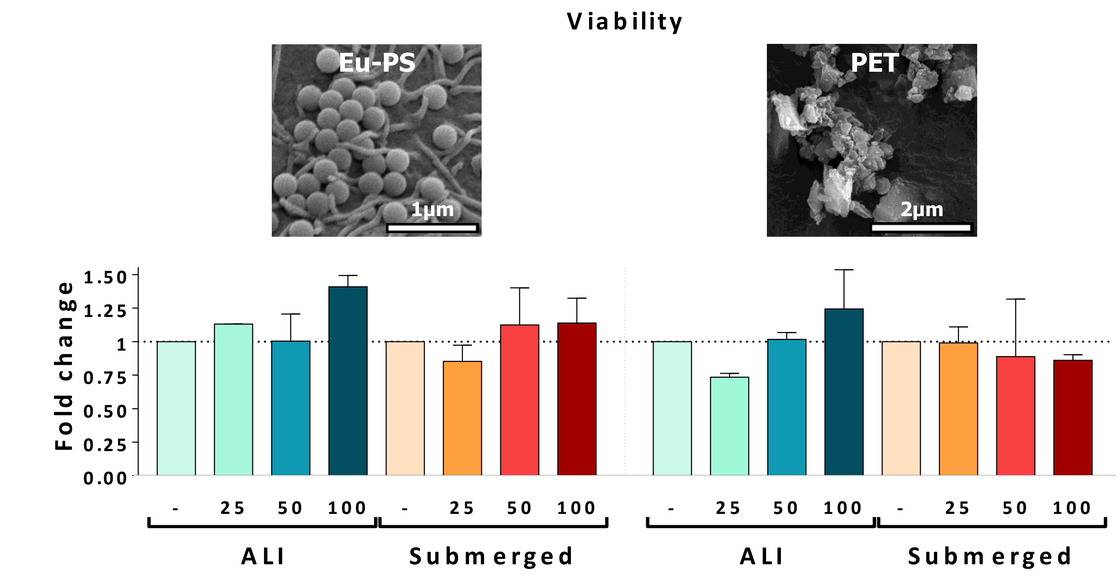


Figure 4: *(Top) SEM image of tested MPs. (Bottom) Cell viability measured via resazurin assay after 48 h of incubation with 25, 50 and 100 µg/ml of MPs.*

Conclusion and outlook: The data presented here show that A549 cells cultured under ALI conditions in plastic inserts with 3 µm pores form a proper epithelial barrier with increased production of surfactants and occludins compared with the same cells cultivated in submerged conditions. Overall, this suggests that ALI cultures may be ideal for testing the effects of MPs on barrier integrity and functionality. In this regard, preliminary results indicate that PET and Eu-doped PS MPs might increase cell viability/metabolic activity in ALI cells in comparison to controls when 100 µg/ml MPs are used. In the future, the effect of MPs on A549 cells will be further investigated using cytotoxicity assays, ultrastructural analysis, barrier integrity analysis and surfactant composition analysis.

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