ENGINEERING SPHERICAL MEMBRANES FOR INHALATION TESTS IN THE PRESENCE OF SYNTHETIC MUCUS

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Introduction

Inhalation tests are fundamental for assessing drug and substance absorption. Standard in-vitro models are based on flat 2D semipermeable membranes at the air-liquid interface. Only recently some studies attempted to replicate the spherical alveolar geometry [1, 2] or the presence of a mucus layer [3]. However, they still lack lung properties such as stretchability. To this end, we developed spherical membranes which replicate the alveolar architecture in a more accurate manner.

Methods

2D and 3D Agarose-Gelatin (AgGel) membranes were fabricated by cast-drying 1%-5% w/v agarose-gelatin solutions in custom moulds. Mechanical tensile tests were performed at a constant strain rate (0.2 s^{-1}). A549 cells were seeded (100.000/cm^2) on the membranes and in PET Transwells as control. Transcellular and paracellular transport was investigated with FITC-dextran and rhodamine. Transepithelial electric resistance (TEER) and Alkaline Phosphatase (ALP) release were also evaluated. The spherical membranes were then interfaced with fluidic compartments (Fig.1) fabricated by stereolithographic printing. Different artificial mucus formulations based on alginate with high and low molecular weight (Alg H and L) and pectin were characterised with a Brookfield viscosimeter to mimic mucus rheology. Neutrophils (1.4 million/mL), derived from healthy donors, were encapsulated in the solutions or suspended in medium as control. Their viability was assessed with the Alamar Blue assay.

Results

The membranes resulted highly transparent and elastic in the range of pathophysiological strains (5-17%) [1] with an apparent elastic modulus = 1.07±0.35 MPa and failure stress = 0.13±0.03 MPa. Fig. 1 shows that cells adhered forming an uniform monolayer on AgGel membranes. Moreover, AgGel3D presented lower TEER, FITC and ALP values with respect to AgGel2D and PET controls (Fig. 2). Alg H was selected for its ability to mimic mucus rheology at low concentrations [3]. In this condition, the viability of encapsulated neutrophils was equal to 60.12±6.09% with respect to controls. Preliminary tests assessed the suitability of the device to mimic dynamic flow and breathing conditions.

Discussion

Results suggested that curve substrates provide physiological culture conditions for lung epithelial cells. Indeed, in conventional cultures, A549 typically presents not-physiological high TEER values and ALP is known to be overexpressed in cancer [3,4]. The fluidic device can be also connected in different configurations replicating lung hierarchical structure and allowing the study of substance passage and deposition from a cellular to an organ level. Moreover, the feasibility of encapsulating patient-derived immune cells in synthetic mucus may increase the relevance of the model. Indeed, it is know that they can affect substance absorption and mucus viscosity due to the formation of neutrophil extracellular traps during inflammation [3].

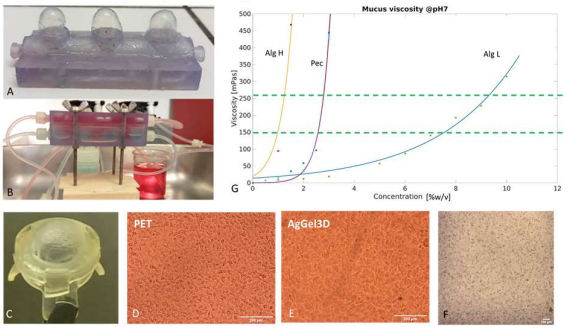

Fig.1 Spherical membraned interfaced with A-B) fluidic compartments and C) cell-crown inserts. D-E) A549 cells on PET and AgGel3D membranes, F) Neutrophils in synthetic mucus, G) viscosity of different materials as a function of their concentration. Green dotted lines indicate pathophysiological viscosity range.

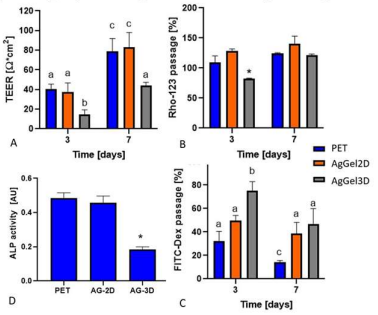

*Fig. 2: A) TEER measurements, B-C) FITC and P-gp passage, D) ALP release. Different letters and * indicate statistical differences ($p<0.05$)*

Conclusion

This work presents preliminary results toward the definition of multiscale human-relevant in-vitro inhalation systems alternative to animal tests. Further tests are on-going to refine the model and investigate substance absorption in dynamic conditions and in the presence of immune cells encapsulated in synthetic mucus with different viscosities replicating healthy and diseased conditions (e.g., lung cystic fibrosis).

References

1. Nossa et al, J Tissue Eng, 12:1-28, 2021
2. Baptista et al, Biomat, 266: 120436, 2021
3. Butnarasu et al, Mol Pharm, 19:34, 2022
4. Gaur et al, J Cancer Res, 16(4):839-842, 2020
5. Srinivasan et al, J Lab Aut, 20(2) 107–126, 2015

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