ENGINEERING CURVED MEMBRANES FOR DRUG ABSORPTION TESTS IN THE PRESENCE OF ARTIFICIAL MUCUS

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Introduction

Investigating drug absorption is fundamental for assessing their efficacy. In the case of inhaled drugs, the ability to cross the alveolar barrier is also related to the presence of the mucosal lining. In healthy conditions airway mucus is a fundamental defense against toxins and pathogens, but diseases such as cystic fibrosis may alter mucus viscosity, hindering drug absorption [1].

Currently, standard in-vitro models are based on flat 2D semipermeable membranes at the air-liquid interface. Only recently have some studies attempted to replicate the spherical alveolar geometry [1, 2] or the presence of a diseased mucus layer [3]. However, they still lack lung properties known to modulate translocation such as stretchability and motion.

To this end, we developed transparent spherical membranes which replicate the alveolar architecture in a more accurate manner. They can be integrated in dynamic bioreactors [1] and coated with artificial mucus formulations to increase the relevance of the model for the study of drug absorption in pathophysiological conditions.

Methods

The membranes were fabricated by casting 1% w/v agarose – 5 % w/v gelatin solutions in custom moulds (Fig.1A). After agarose crosslinking (20 minutes at 4°C), the samples were incubated overnight at 37°C with 100 U/g microbial transglutaminase (mTG) enabling gelatin crosslinking. The gels were dried for 24h at 40°C and sterilized under UV for 30 minutes. Finally, the Agarose-Gelatin (AgGel) membranes were rehydrated in deionised water for 1h. Mechanical tensile tests to break were performed at a constant strain rate (0.2 s^{-1}) and the apparent elastic modulus (E_{app}) was derived as the slope of the stress strain curve in the linear region.

A549 cells were seeded on the membranes mounted in CellCrown inserts (100.000 cell/cm^2) and in PET Transwells as control. Transepithelial electric resistance (TEER) measurement (EVOM – WPI) and bright field images (Olympus) were acquired to assess the presence of an intact monolayer. Then, transcellular and paracellular transport was respectively investigated with FITC-labelled dextran (0.5 mg/mL) and rhodamine (10 μM). Pg-p protein activity was calculated as the percentage of rhodamine passage from the basal to the apical compartment.

Results

The membranes are highly elastic in the range of pathophysiological strains (5-17%) [1] with E_{app} = 1.07 ± 0.35 MPa and failure stress = 0.13 ± 0.03 MPa.

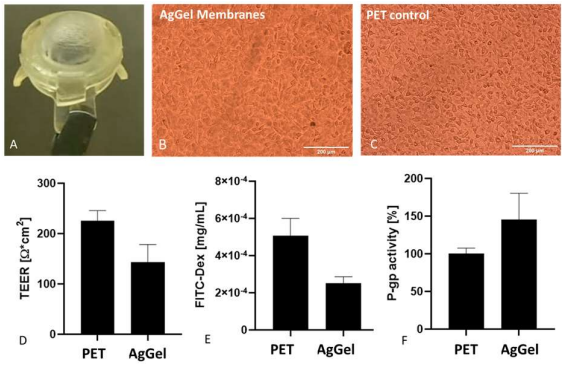

Figure 1: A) Spherical Membrane on inserts; B-C) Brightfield images of A549 cells on AgGel membranes and PET controls; D-E-F) TEER measurement, FITC passage and P-gp activity at day 1.

As shown in Figure 1B, cells adhere and spread on AgGel membranes. Moreover, their performance was comparable with PET controls in terms of barrier tightness and transport features. The lower FITC passage indicated the formation of stronger tight junctions, while the higher Pg-p activity suggested that the membranes are able to promote cell polarization.

Conclusion

Spherical, transparent, and cytocompatible AgGel membranes were fabricated and characterised. Their properties in terms of shape, mechanical behaviour, cell compatibility, and permeability can be exploited for the development of more reliable and human relevant inhalation tests. Further tests are on-going to develop an artificial mucus gel able to replicate healthy and diseased mucus rheology and hence investigate drug adsorption in the presence of mucus and inflammatory cells.

References

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