Aerosolised lung surfactant-based formulation stabilisation to optimise **inhalable** controlled **drug delivery**

By Melih Engur
Supervisor: Dr Jorge Bernardino

Imperial College London

EPSRC Centre for Doctoral Training in Aerosol Science

Alveolar Basics and Surfactant

The alveoli have total surface area of ~70m2 with a respiratory barrier and diffusion distances as thin as 200nm(1,2,3). As demonstrated in *figure 1* surface area exponentially increases with each generation from the trachea, bronchi, bronchioles and finally the alveolar sacs (4, 5).

AT1 cells cover over 90% of alveolar surface area making them the gas exchange region of the lungs(6). AT2 cells secrete lung surfactant containing: surfactant proteins(SP) A,B,C and D and lipids. Surfactant is important as it prevent atelectasis by maintain surface tension(6).

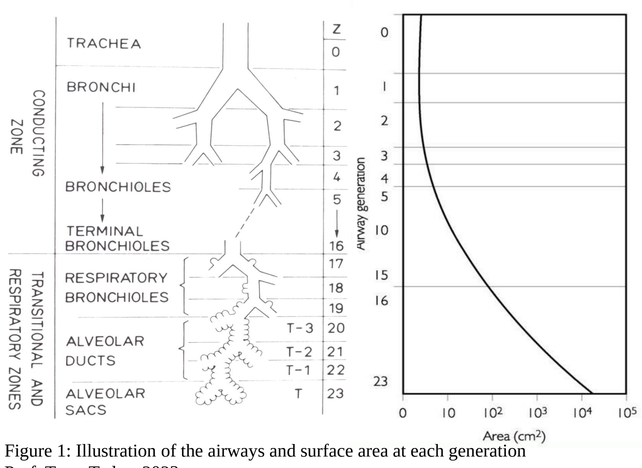

Figure 1: Illustration of the airways and surface area at each generation
Prof. Terry Tatley, 2023

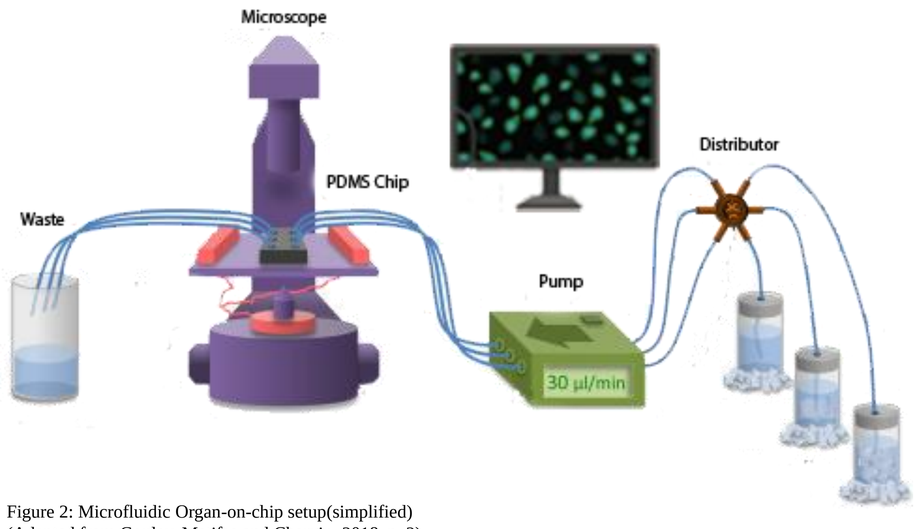

Figure 2: Microfluidic Organ-on-chip setup(simplified)
(Adapted from Goulev, Matifas and Charvin, 2018, p. 2)

Statement of Problem

- Inhalable medicine is currently used to treat condition like COPD and asthma, however, these are not examples that demonstrate distal alveolar deposition(7).
- During the pandemic, nebulised delivery of LS lipids for COVID-19 patients showed favourable pharmacokinetics, demonstrating alveolar deposition(8).
- Later studies created LS based liposomal lipid nanoparticles for drug delivery with poor success due to reasons such as hygroscopic aggregation, instability during aerosolization, protease action and immune response(7-10).
- Identifying the optimal LS based liposomal nanoparticle formulation has the potential to combat these issues and undergo epithelial uptake(10, 11).
- One example is the coating of the nanoparticle with polyethylene glycol to promote immune-evasion(11)

Objectives

- **Primary Objective:** Design LS-based aerosolized liposomal lipid formulations that reach deep into alveolar ducts and are internalized by AT1 cells.
- Secondary objective: Observe how different formulations of liposomal nanoparticles enter AT1 cells and quantify their sensing by cell membrane receptors using advanced fluorescent microscopy and imaging techniques*(figure 3)*.
- Tertiary objective: Perform a comparative analysis of promising formulations with oncotherapeutic molecules provided by industrial collaborators Akamis Bio Ltd using the OOC model*(figure 2)*.

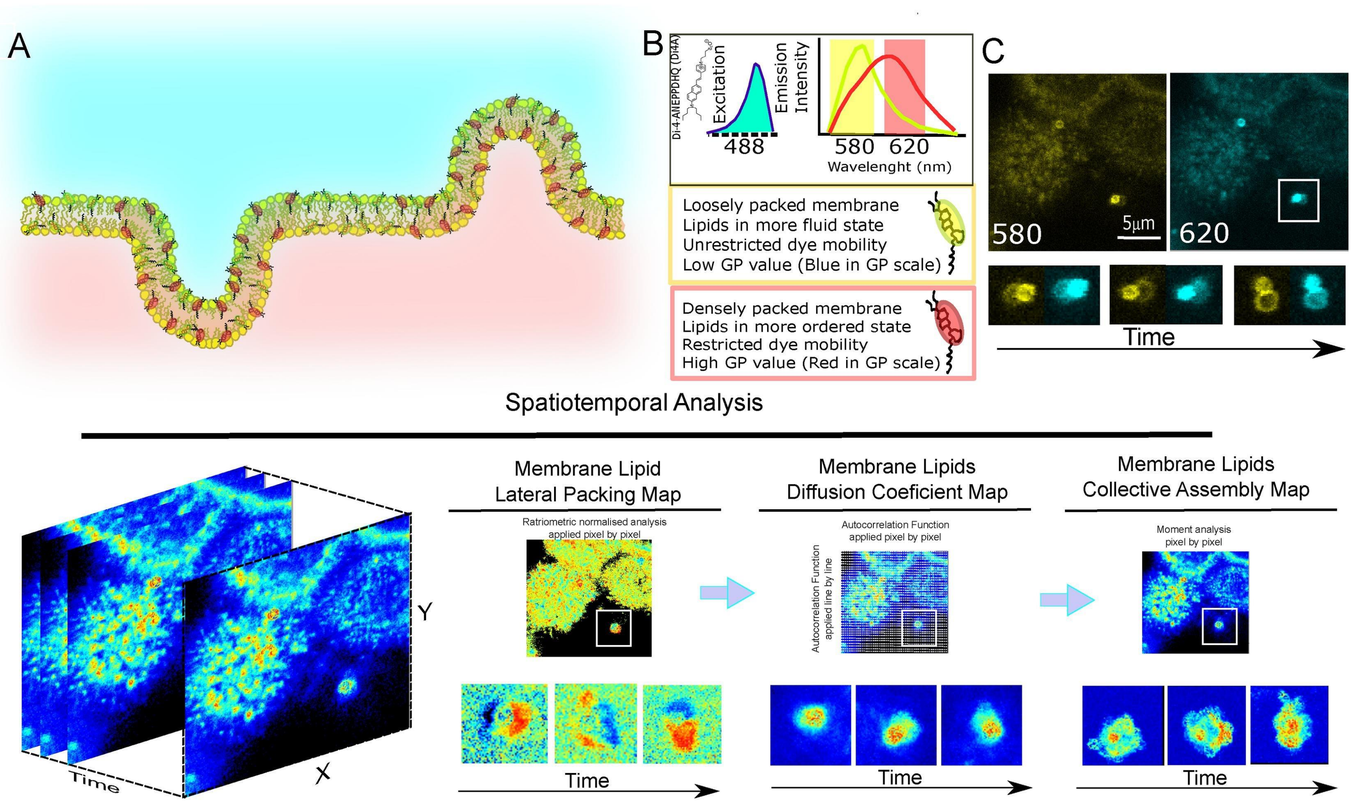

Figure 3: RICS and GP Analysis for Membrane Density and Diffusivity Analysis
(Bernabé-Rubio M et al, 2021)

Methodology

This study will use hAELVi cells which express tight cell junctions critical for air-lung modelling(12). We will deploy organ-on-chip (OOC) model to mimic in-vivo conditions (13). Customised PDMS chips will be developed within the lab seen in *figure 4.* Fluorescent microscopy to visually confirm uptake of nanoparticle will be used by tagging liposomes with fluorescent markers. LS based liposomal lipid nanoparticles will be formulated, continuing from preliminary lab findings, with differing properties to identify optimal internalisation and stability.

STED Inverted Confocal Microscope will be used to analyse cell response to formulations. For this, a solvatochromic dye (Di4A) will be applied onto cells which is quickly move into the cell membrane(13). The emission spectra of this dye varies depending on membrane fluidity which will be used to identify cell sensing and cell membrane response to different formulations (13).

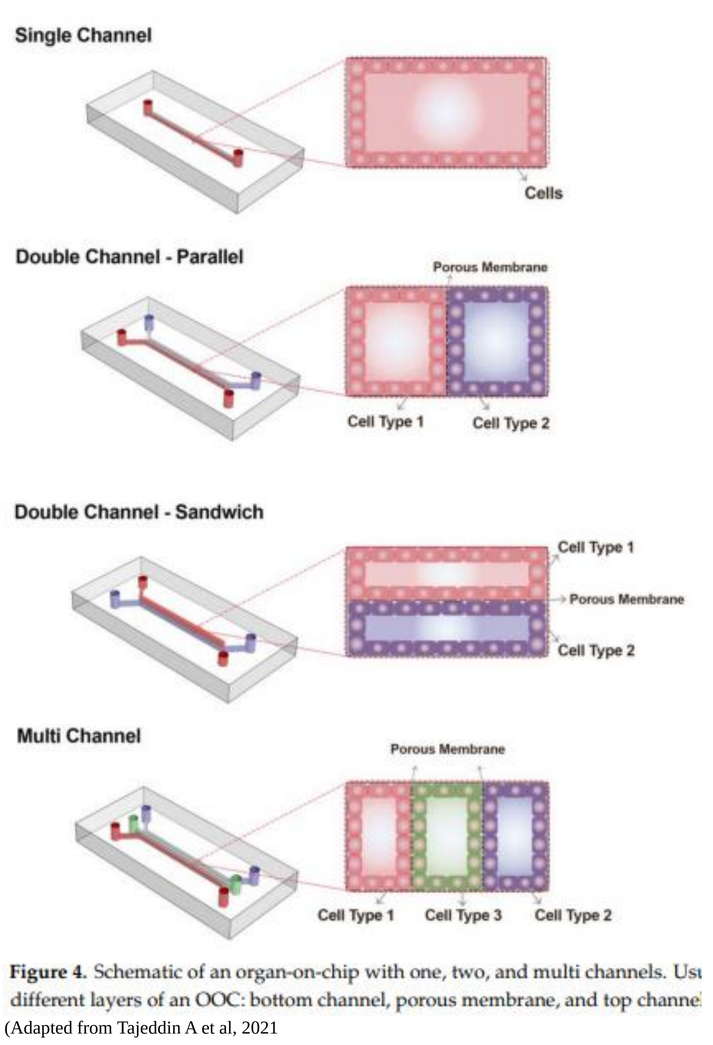

Figure 4. Schematic of an organ-on-chip with one, two, and multi channels. Us different layers of an OOC: bottom channel, porous membrane, and top channe
(Adapted from Tajeddin A et al, 2021

Conclusion

In conclusion, this study will deploy OOC model and advanced imaging techniques to assess varying formulations to optimise pulmonary alveolar-epithelial drug delivering. This project extends to a placement with our industrial partner AkamisBio Ltd focusing on clinical-stage tumour gene therapies for varying cancers.