Direct quantification of transendothelial electrical resistance to estimate the barrier function in organ-on-chip embedded with double porous membrane

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ABSTRACT

Organs-on-chips are microfluidic cell culture systems that allow studies of cell-cell interactions and functional analysis of cultured tissues. When cell monolayers like endothelium or epithelium are cultured in organs-on-chips, one of the key aspects to evaluate is barrier function. Methods to evaluate barrier function in organs-on-chips are characterization of flux and transendothelial electrical resistance (TEER) [1, 2].

We have developed a bio-artificial membrane with double-porous morphology[3] containing (i) surface macrovoids that could be easily accessible for cell proliferation and differentiation; (ii) a macroporous spongy network to transfer essential nutrients, gas, growth factor between the macrovoids and the supernatant and also to provide sufficient adhesion between the cell-matrix interface, based on a poly (ε-caprolactone) (PCL) and Chitosan (CHT) blend. We are planning to evaluate the barrier function of cell layers (lymphatic endothelial cells) on these membranes by employing an organ-on-a-chip device with integrated electrodes **(Fig. 1)**.

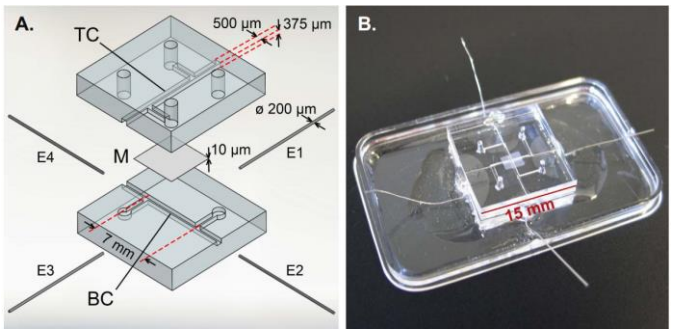


Fig. 1. *Organ-on-chip devise. (A). Exploded view of the PDMS chip with the top channel (TC), membrane (M), bottom channel (BC) and platinum wire electrodes (E1, E2, E3, E4). The culture area A on the membrane suspended between both channels is 0.25 mm^2 (Ref. 1, 2).*

The membranes were developed by a modified version of the conventional liquid-induced phase inversion method by diffusion between solvent as formic acid/ acetic acid mixture and non-solvent NaOH solution. PCL and CHT with a ratio 100/0, 90/10, 80/20 and 70/30 were prepared. The casted solution was covered by an isoporous PET macrovoid-generator membrane of pore diameter 10 µm to achieve the double porous structure. The area where the polymer solution was covered by the macrovoid-generator membrane, two different solvent exchange rate was observed leading to the formation of double-porous structure. The macrovoids were formed where non-solvent has a direct access with the polymer solution through the pores of the macrovoid-generator membrane. In addition, the interconnected macropores were formed where the non-solvent could not make a direct connection with the polymer solution. After preparation, membranes were subject to determine their flux properties in Amicon cells with real time flow rate. SEM images of the cross section of the scaffolds confirmed that the macrovoids are connected with each other through the spongy macroporous network.

The organ-on-chip device was previously developed by Van der Helm, *et al*. and tested with membrane patches based on polycarbonate[1, 2]. The device consists of two polydimethylsiloxane (PDMS) slabs where the membrane patch is embedded between two cross-flow channels (width × height, 500 µm × 375 µm) equipped with integrated platinum electrodes **(Fig. 1.)**[1, 2]. We are currently embedding our double-porous membranes in the devices, and are setting up cultures of human dermal lymphatic endothelial cells in the top channel. We intend to measure barrier function by TEER by assessing the resistance between the four electrodes as follows[1, 2].

$$TEER = A_{cult} \cdot R_m = A_{cult} \cdot \frac{1}{4}\left(R_{1\to2} + R_{1\to4} + R_{2\to3} + R_{3\to4} - 2R_{1\to3} - 2R_{2\to4}\right) \left[\Omega\ cm^2\right] \quad \textbf{(1)}$$

Where $\mathbf{A_{cult}}$ is culture area (0.25 cm^2), $\mathbf{R_m}$ is the average resistance of the valid combination of the four electrodes (E1, E2, E3, E4) and $\mathbf{R_{1\text{-}2}}$ is the resistance between E1 and E2.
Flux can be determined in the same organ-on-chip device without any electrode but by attaching a capillary tube to the bottom channel where the permeate was collected through the capillary tube with time.

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ACKNOWLEDGMENTS The author would like to thank the Research Frontiers Programme Erasmus Mundus Doctorate in Membrane Engineering (5th ed) – EACEA for providing financial support to the project.