A sensorised, microfluidic Ussing chamber manufactured by stereolithography and soft lithography techniques

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Abstract. Motivated by the need for improved platforms in biomedical research, this study addresses challenges associated with traditional Ussing chamber systems, widely used in studying biological barriers like the epithelial barrier of the gut. These challenges include complexity, high sample volumes, and limited compatibility. By combining stereolithography and soft lithography techniques, a microfluidic Ussing chamber is manufactured, overcoming these limitations, and incorporating compatibility with microscopy. Validation through Trans-Epithelial Electrical Resistance (TEER) measurements confirms its efficacy in assessing ion permeability dynamics, utilizing Caco-2 cell monolayers. The study showcases the capability of the manufactured chamber for sensing impact of calcium on tight junctions.

1 Introduction

Microfluidics, an interdisciplinary field that is quickly developing at the nexus of physics, engineering, chemistry, and biology, has revolutionized the way small volumes of fluids can be manipulated and analysed. At its core, microfluidics deals with the precise control and manipulation of fluids at the microscale, typically within channels with dimensions ranging from tens to hundreds of micrometres.

One of the growing fields in microfluidics is the study of biological barriers, which are essential to ensure the proper organism function. In particular, the epithelial barrier of the gut controls the absorption of nutrients, water, and limits the invasion of bacteria [1]. One platform to study this barrier is the Ussing chamber, a device composed of two fluid compartments separated by a permeable membrane, typically an epithelial tissue mounted in between. By applying an electric potential across the tissue and monitoring the resulting current, researchers can investigate barrier functions.

The original design of the Ussing chamber system is rather intricate and not particularly user-friendly, utilizing a high volume of sample and lacking compatibility with the widely used multiwell plate format. The aim of this study was to develop a microfluidic Ussing chamber, making it easier to use, reducing the required volume of reagents, and offering compatibility with microscopy, perfusion and parallelisation.

For this, stereolithography (SLA) was used to manufacture the masters of the Ussing chamber. This is an optical additive manufacturing technique that relies on the selective polymerization of photosensitive resins through exposure to an ultraviolet laser, allowing for the construction of 3-dimensional (3D) structures with extraordinary precision. Following SLA, masters have been replicated using soft lithography of polydimethylsiloxane (PDMS), broadly used by its flexibility, biocompatibility and optical transparency.

In this work, SLA and soft lithography have been used to manufacture a highly functional microfluidic Ussing Chamber, successfully validated through Trans-Epithelial Electrical Resistance (TEER) measurements.

2 Materials and Methods

2.1 3D Printer

A Form 3B SLA 3D printer was used to produce the masters. This printer features a UV laser with λ=405 nm, and a power of 250 mW and offers an XY resolution of 25 μm. The laser spot size is 85 μm. Resolution on the Z axis for the commercial Clear V4 resin is 25 μm. Printed masters consisted of 8×8×4 cm^3 cubicles. Additionally, a pillar that fits into the bottom of each cubicle to form the internal chamber was also printed. Furthermore, auto-aligning structures were added to assemble both parts. All structures were designed using the Computer Aided Design (CAD) software Fusion 360.

2.2 PDMS soft lithography

The PDMS was prepared from Sylgard 184 elastomer by mixing the monomer and the curing agent in a ratio 10:1.

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The mixture was then filled inside the masters. Then, devices were introduced in a vacuum chamber for 40 min at 400 mbar to remove bubbles produced during the mixing process and cured in an oven for 12 h at 60 ºC.

3 Results and Discussion

3.1 Device manufacturing and cell culture

The manufacturing process starts with the printing of both master and pillar (Fig. 1a), which are designed to interlock seamlessly. Subsequently, 23G needles (0.6 mm in diameter) are carefully inserted into the lateral holes of the master (Fig. 1b), followed by pouring of uncured PDMS.

Once cured, the pillar and needles are extracted, resulting in the formation of a chamber and the fluid/electrode ports, respectively (Fig. 1c). Two identical PDMS slabs are bonded together resulting in a top and bottom chamber, which are separated by a microporous membrane. Bonding is achieved by O_2-plasma activation and an O-ring is used to hold the porous membrane in place and avoid leakage (Fig. 1d). The two fluid/electrode ports on either side allow each for the introduction of liquid flow, and electrodes, used to measure TEER.

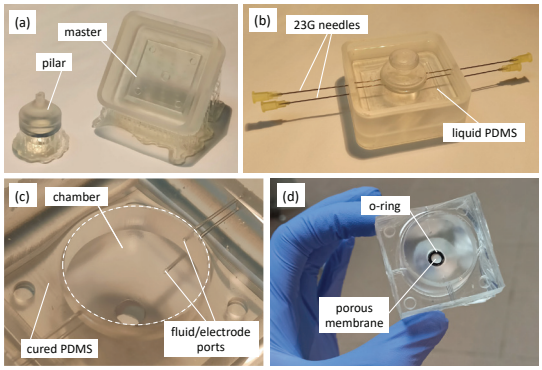

Fig. 1. Manufacturing process of Ussing Chamber. First, a) the master and the pillar, which can be coupled, are printed. Next, b) 23G needles are inserted and the master is filled with uncured PDMS. After curing, the pillar and needles are removed forming c) the chamber and the ports, respectively. Finally, d) both halves are bonded, featuring an O-ring to fix the porous membrane in between.

In parallel, Caco-2 cells were grown in MEM supplemented with 20 % FBS, 1 mM Na-pyruvate, 100 $U \cdot mL^{-1}$ penicillin and 100 $\mu g \cdot mL^{-1}$ streptomycin (1 % Pen/Strep). To form an intestinal epithelium, cells were seeded at 10^5 cells/cm^2 and grown in 12-well semi-permeable inserts (transwell), featuring PET membranes of 1 µm pore size. The membranes were cut using an 8 mm punch and sandwiched between the two chambers, leaving an exposed area of 1.12 cm^2.

3.2 TEER measurement

The integrity of the Caco-2 monolayer was assessed by introducing Ag/AgCl electrodes through the device ports (Fig. 2a) and measuring TEER by connecting to a Epithelial Voltometer EVOM^2 from Millicell (Fig. 2b). The TEER ($\Omega \cdot cm^2$) was calculated by subtracting the readings of cell-free inserts from the cell cultured inserts and multiplying by the surface area of the membrane (all measurements were done in duplicate).

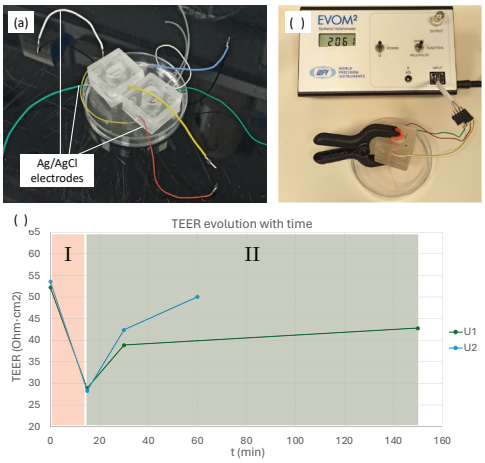

Fig. 2. a) Two manufactured Ussing chambers with the electrodes and wires coupled, allowing the connection to a b) epithelial voltometer. c) TEER behaviour over time in presence of chelators.

Knowing the central role of calcium in the establishment of cell adhesions, the use of divalent cation chelators will disrupt tight junctions, increasing ion permeability and decreasing trans-epithelial resistance. Fig. 2c shows the evolution of the TEER over time inside the manufactured chamber. Basal conditions were performed with live cells in HBSS with Ca^{2+} and Mg^{2+}. Then, HBSS without Ca^{2+} and Mg^{2+} and with 2mM EDTA was introduced for 15 mins to induce a temporary barrier dysfunction. Subsequently, HBSS with Ca^{2+} and Mg^{2+} was reintroduced to simulate a recovery step. The Ussing chambers showed a 48% decrease in TEER for the first 17 mins (Region I), followed by an increase (Region II), related with the re-introduction of Ca^{2+} and Mg^{2+} ions, validating the capacity of our Ussing chambers to make time-resolved TEER measurements.

4 Conclusions

The manufacturing of a microfluidic Ussing chamber using a combination of stereolithography and soft lithography methods was demonstrated. The observed changes in TEER following calcium depletion and restoration validated the chamber capability to accurately assess ion permeability dynamics. Overall, this study underscores the importance of advanced manufacturing methods in developing innovative tools for biomedical research.

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