Towards reliable *in vitro* models – New bi-compartmental platforms for replicating tissue barriers

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Abstract

In vitro cell cultures represent a widespread methodology for pathophysiology studies with a high benefit/cost ratio. We have developed and are validating TToP, a novel, versatile, and scalable culture system allowing compartmentalized cell/tissue cultures with the aim of mimicking the human pathophysiological microenvironment of tissue barriers.

Introduction

Nowadays, drug discovery and development processes are extremely long, expensive, and inefficient.[1] Existing in vitro preclinical models are based on oversimplified mono compartmental cell cultures.[2] On the other hand, animal models show differences that do not always allow a direct translation to humans. Micro-Physiological systems (MPSs) provides the basis for preclinical assays with greater predictive power with respect to traditional cell cultures, and with the ambitious perspective to replace animal models in compliance with 3R's principles. However, PDMS-based organ-on-chip technologies translation to commercial products is very limited, due to high costs and hurdles for large scale manufacturing.[3] For these reasons, CO_2 laser micromachining of plastics and use of double-sided tapes could be considered as a valid alternative for MPS prototyping, allowing good reproducibility, and rapid and easy-to-handle production processes. With this fabrication strategy in mind, we developed TToP, a novel acrylic-double-sided tape in vitro culture system,[4] compatible with 12-well plates and enabling compartmentalized in-vitro cell/tissue cultures and co-cultures. We introduced a new additional feature that allows the recover/reuse of the biological sample maintaining the morphological structure and vitality without any damage potentially due to manipulation and enabling the direct access to the biological sample for microscopy (epifluorescence and confocal) and qRT-PCR.

We also optimized fabrication processes to achieve a repeatable and reliable production of 70 systems simultaneously, with a low attrition rate. A biological validation has been carried out in two case studies, seeding Caco-2 epithelial cells and EAhy-926 endothelial cells and evaluating confluence and differentiation.

Materials and Methods

Acrylic and double-sided tapes were used and laser cutting (Versa Laser V2.30 ULS) procedures optimized, to develop a device suitable to house commercial polymeric microporous membranes and perform bi-compartmental static cell cultures, over a culture surface equal to a 96-well plate. As first step of the biological validation of the culture system, Caco-2 cells (ATCC®HTB-37™), a human cell line recognized as a model of colonic epithelium, were seeded on Whatmann Nucleopore® (pore size 5 µm, $4x10^4$ cells/system) polycarbonate microporous membranes and cultured in static conditions using Dulbecco's Modified Eagle Medium (DMEM) with 10% FCS, 1% Pen-Strep, 5% Gentamicine, in a 37°C, 5% CO_2 standard incubator. Confluent cells fixed in 0.8% paraformaldehyde PF) were stained by DAPI (nuclei), and by differentiation/lineage-specific antibodies (Human Epithelial Antigen - HEA, Junction Adhesion Molecule - JAM).

Then, EAhy-926 cells (ATCC®CRL-2922™) a recognized model of vascular endothelium, were seeded on it4ip ipCELLCULTURE™, (pore size 3 µm, $3x10^4$ cells/system) polycarbonate microporous membranes and cultured in static conditions using DMEM with 4.5 g/L D-Glucose, L-glutamine and sodium pyruvate, with 10% FBS, 1% Pen-Strep, 1X HAT (hypoxanthine-aminopterin-thymidine) in a 37°C, 5% CO_2 standard incubator. At day 7 cells were stained by DAPI (nuclei), and differentiation/lineage-specific antibodies (CD-31, VE-CAD). Moreover, EAhy-926 cells monolayer gene expression levels of TP53 and VEGF-A have been evaluated by qRT-PCR and compared with Transwell® inserts.

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Results

The TToP manufacturing processes have been optimized to scale-up the production, obtaining series of 70 devices simultaneously, with high reproducibility (Figure 1). In parallel, assembling protocols and accessories to speed up and standardize the membrane mounting procedure were refined.

In the first set of experiments (Figure 2 A, B) Caco-2 confluent monolayer polarization was observed by confocal microscopy after the retrieval of the membrane from the system, as presence of expressed JAM at the apical side and of HEA at the basal side, respectively.

In the second set of experiments (Figure 2 C, D) EAhy-926 cells phenotype (CD31/PECAM, VE-CAD), gene expression (VEGF-A, TP53) and confluence (DAPI) were observed showing outcomes comparable and sometimes superior to that obtained with Transwell® inserts.

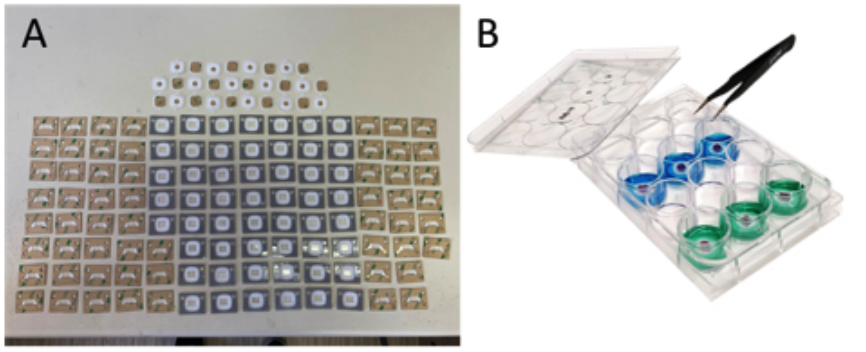

Figure 1. A) Representative image of TToP systems, resulting from a single optimized production process. B) TToP systems in 12-well plate, ready for use.

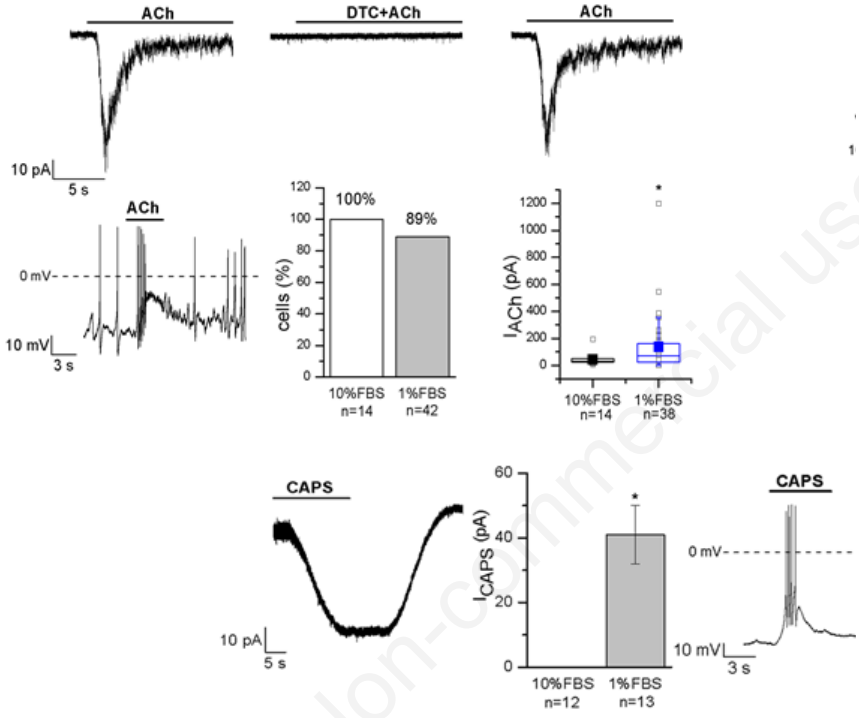

Figure 2. A) Confocal cross-section of Caco-2 cell monolayers labelled with HEA DAPI and B) JAM and DAPI, demonstrating cell polarization. C) Confocal images of EAhy-926 cell monolayers labelled with DAPI, CD-31 and VE-CAD. D) VEGF-A gene expression comparison TToP vs. Transwell®.

Discussion and Conclusions

The optimization of TToP fabrication processes and assembling protocols led to a highly reproducible and scalable system. Moreover, we obtained confluent, differentiated and polarized monolayers with high reproducibility.

In perspective, we will focus on recreating *in vitro* controlled biomechanical vessel-like cues, such as hydrodynamic stimuli, 3D extracellular matrix and cell-cell interactions (Endothelial cells – Smooth Muscle Cells), to mimic vascular Carrier functions. This model will be exploited to study pathophysiological conditions, drug safety/toxicity effects and in perspective will be coupled with other models, to study multi organ interactions.

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