XIII JORNADA DE JÓVENES INVESTIGADORES/AS DEL I3A

IMPROVING CELL-TO-CELL AND CELL-TO-MATRIX CONTACT INSIDE MICROPHYSIOLOGICAL SYSTEMS

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INTRODUCTION

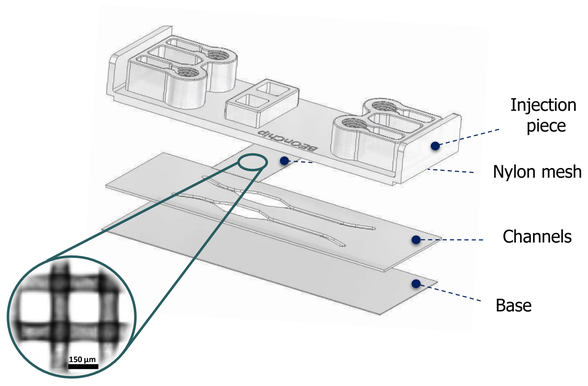
The appearance of the microphysiological systems has allowed, with their channel and chamber designs, to resemble the histological distribution *in vitro* faithfully. One of the main limitations of this technology is the presence inside the microfluidic chips of inert materials separating two different compartments. Those inert materials avoid direct contact between cells and matrix but also introduce a rigid structure in the model affecting the physiological cell behaviour.

To address this issue, two microfluidic devices have been developed that minimize the presence of inert materials within the chip, enhancing cell-to-cell and cell-to-matrix interactions. Biological validation includes cell migration assays and epithelial development, contributing to more biomimetic *in vitro* models.

THE MESH

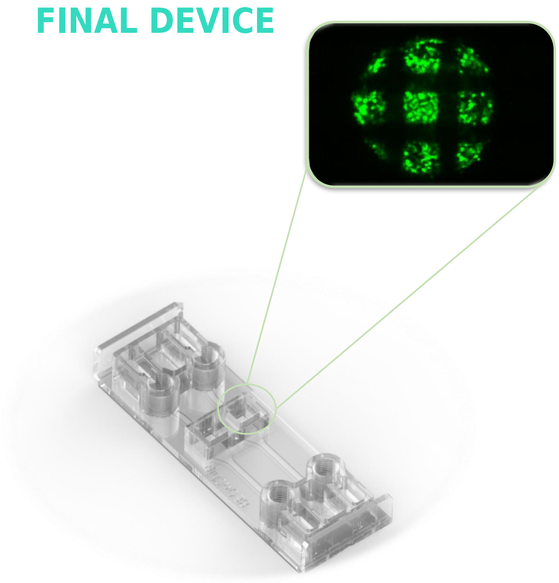
FABRICATION TECHNIQUE

COMPONENTS



Mimicking the cell-cell interaction without any hydrogels. This chip contains a **nylon mesh** with regular **pores of 150 microns** to achieve this.

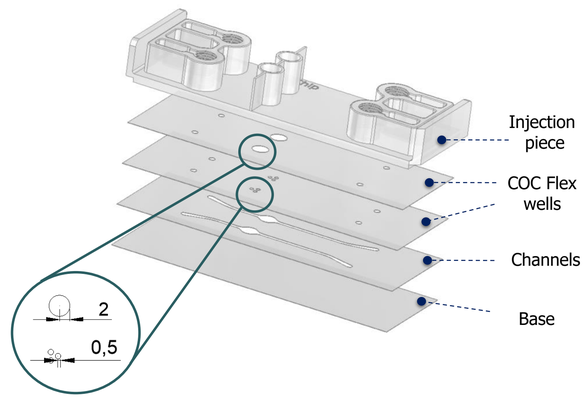
FINAL DEVICE



MACROPORE

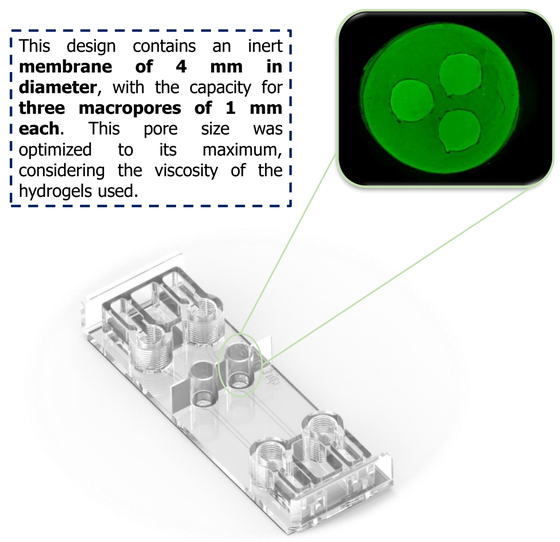
FABRICATION TECHNIQUE

COMPONENTS



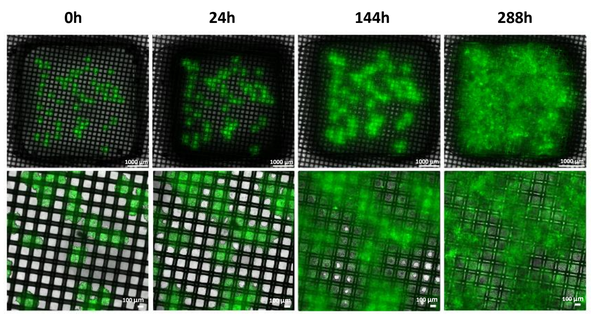
FINAL DEVICE

This design contains an inert **membrane of 4 mm in diameter**, with the capacity for **three macropores of 1 mm each**. This pore size was optimized to its maximum, considering the viscosity of the hydrogels used.

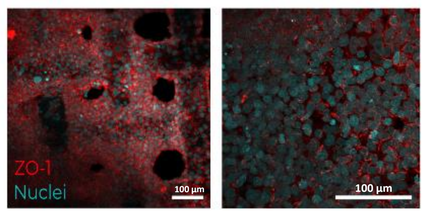


BIOLOGICAL VALIDATION

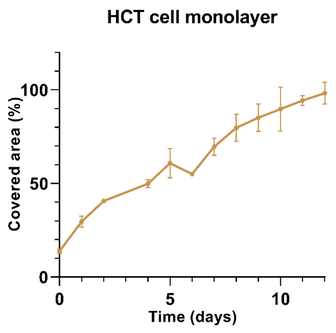
Epithelium generation



Top-down perspective of the **evolution of the HCT-116 monolayer** at different time points (0, 24, 144, and 288 h).

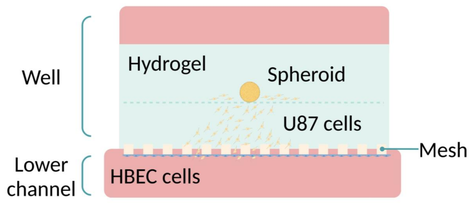


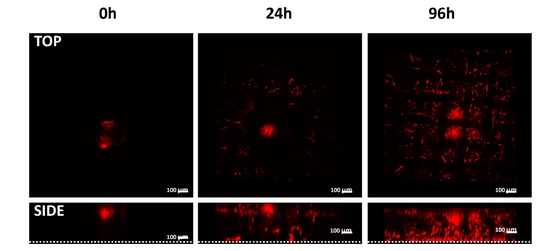
Immunolabelling of the **tight junctions** with **ZO-1 (red)** and **nuclei** counter labelling with **Hoechst (cyan)**.

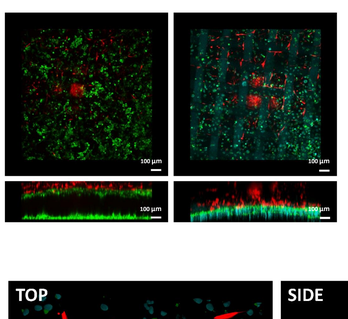


Evolution of the covered area (%) of the HCT cell monolayer on the nylon membrane over time.

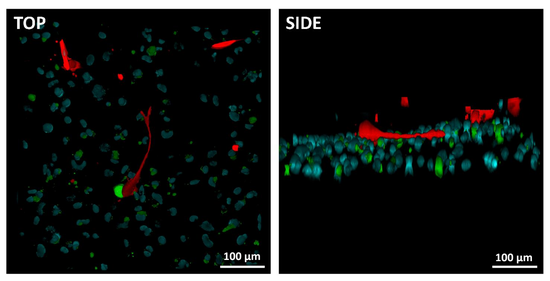
Migration assay and endothelium generation







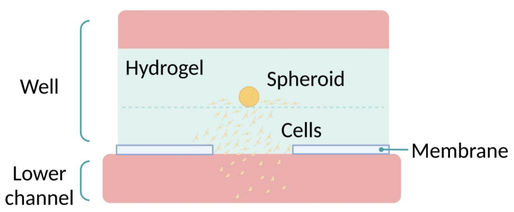
Confocal imaging captures the invasion of **U-87 MG cells** towards the perfusion channel over 96 h, alongside their interactions with **endothelial HBEC cells** during the 24 h period at 120 and 144 h.

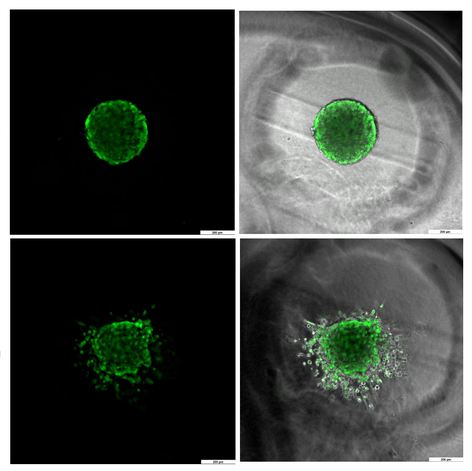


Z-stack reconstruction illustrating interactions between **U-87 MG cells (in red) and endothelial cells (stained in green with their nuclei counterstained in cyan)**.

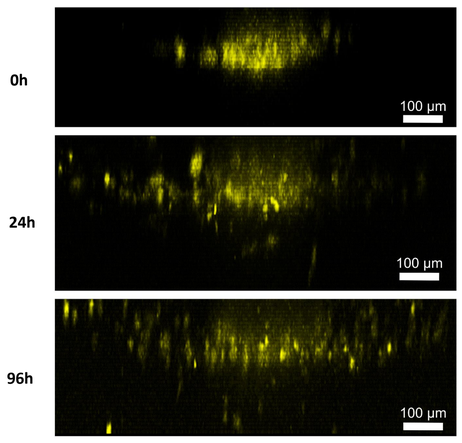
BIOLOGICAL VALIDATION

Migration assay



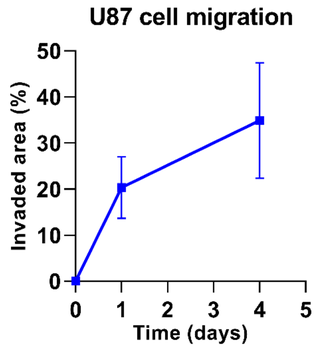


U-251 spheroid in the center of one of the holes.

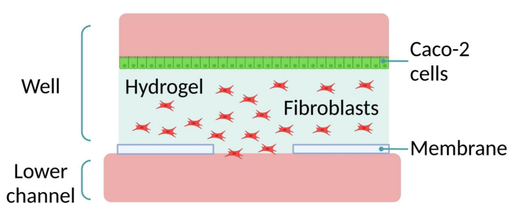


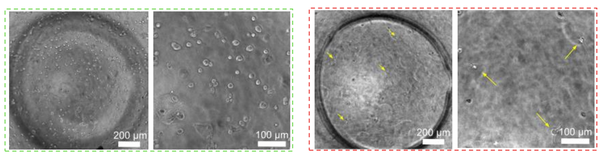
Lateral view of the **migration** along the days with **U-87 cells** artificially colored in yellow.

Evolution of **U-87 cell migration** within the Macropore device over successive days, quantifying the **invaded area as a percentage**.

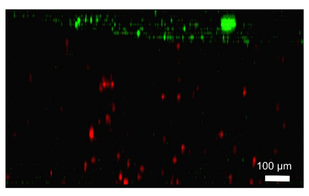


Co-culture assay





Caco-2 Fibroblasts



Fibroblasts (red) were **embedded** within a **collagen hydrogel** and introduced into the well. Following this, **intestinal epithelial cells (green)** were **seeded onto the hydrogel**, with media added on top.

CONCLUSIONS

Innovative devices have been developed to reduce the presence of inert materials within microphysiological systems, thereby enhancing cell-to-cell and cell-to-matrix contact.

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