Intestine-On-Chip: Enhancing *In Vitro* Intestinal Models Using Caco-2 and HT29-MTX Cocultures Under Different Flow Conditions

Rut López [1,2], Clara Bayona [1,2], Ignacio Ochoa[1,2,3], Estela Solanas[1,2]

[1] Tissue Microenvironment (TME) Lab. University of Zaragoza, C/ Mariano Esquillor s/n, 500018 Zaragoza, Spain

[2] Instituto de Investigación Sanitaria Aragón (IIS Aragón)

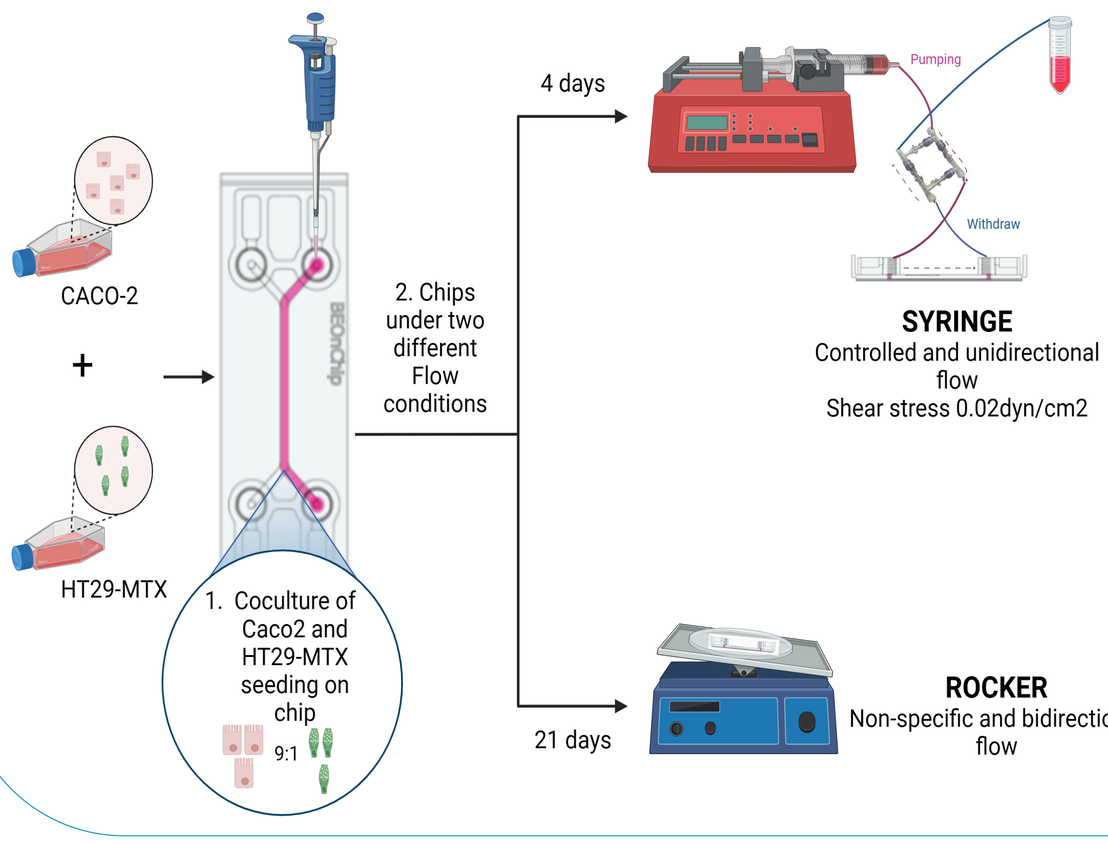
[3] Centro Investigación Biomédica en Red. Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Zaragoza, Spain

INTRODUCTION

The gastrointestinal tract is essential for growth and development, and its dysfunction can lead to various diseases. Research is hindered by ethical and technical challenges, necessitating the use of realistic *in vitro* models. Two-dimensional (2D) and three-dimensional (3D) models with enterocytes and goblet cells have been created to simulate the intestinal barrier. Advanced microfluidic systems and organ-on-a-chip devices are a promising tool for studying the gut barrier and nutrient absorption, but they need to be improved to better mimic physiological conditions. For these reason, our study focuses on enhancing these models by developing a microfluidic device to study intestinal permeability under different flow conditions.

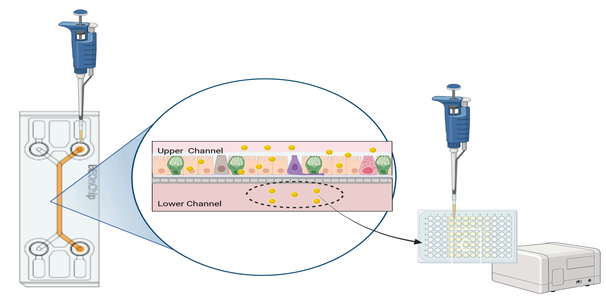
METHODS

Intestine-On-chip Model



Permeability assay

To determine the barrier integrity, permeability was analyzed using the Lucifer Yellow method on days 0, 7, 14 and 21 for the rocker and 0 and 4 for the syringe pump.



Fluorescent labelling

Chips were fixed in 4% paraformaldehyde and stained with Rhodamine-Phalloidin and DAPI markers to determine the expression of F-actin and nuclei.

Statistical Analysis

All experiments were performed at least three times and statically analyzed with Prism 7. P values ≤0.05 were considered statistically significant.

RESULTS

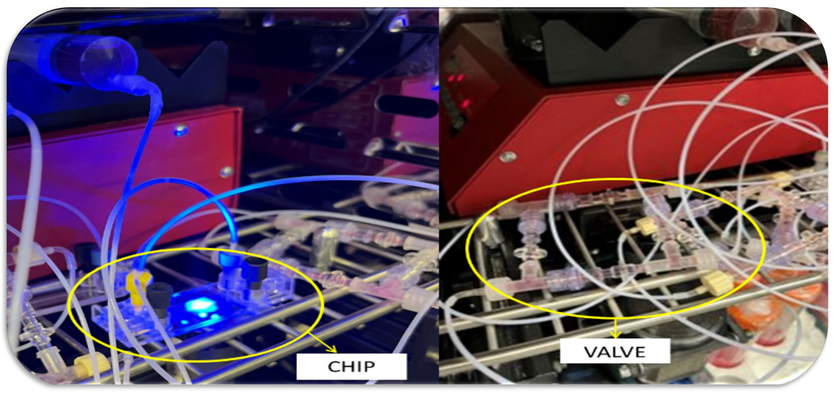


Figure 1. System design of our intestine-on-a-chip model attached to a syringe pump with valve.

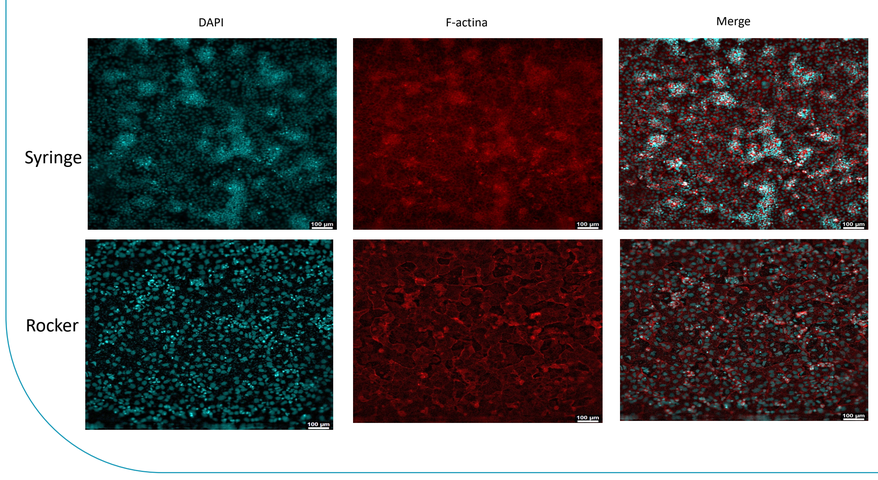


Figure 2. Microscopy of our intestine-on-a-chip model at day 4 showing the cell monolayer by F-actin labelling and nuclei, cells were arranged in a single layer adjacent to the basement membrane.

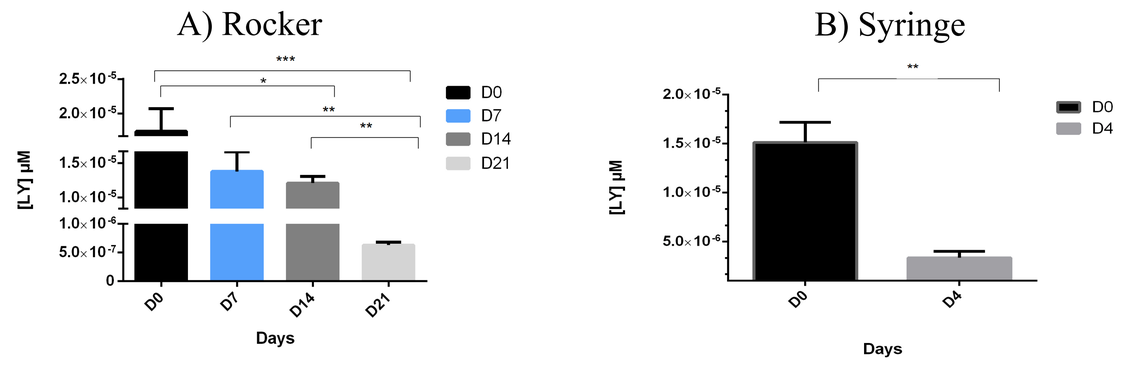


Figure 3. Mean and standard error of the permeability values (n=3) of the co-culture in the microfluidic device. A) With a non-specific flow at day 0, 7, 14 and 21. B) With the syringe pump at day 0 and 4. (*** p <0.001, ** p< 0.01, * p < 0.05)

Decreased Permeability Under Bidirectional and non-specific Flow

Chips subjected to non-specific and bidirectional flow exhibited a statistically significant decrease in permeability from day 14 of culture compared to the initial monolayer, reaching its lowest point after 21 days in culture ($p \leq 0.001$).

Impact of Shear Stress on Permeability in controlled flow conditions

Coculture with controlled and unidirectional flow, under a shear stress of 0.02 dyn/cm², showed similar permeability values after 4 days compared to coculture under non-specific conditions by rocker in 14 days.

Incorporating a controlled and unidirectional flow can decreased culture time for mimicking *in vivo* intestinal permeability, likely enhancing epithelium differentiation and intercellular junctions.

CONCLUSIONS

Our study shows that the co-culture of Caco-2 and HT29-MTX on chips under controlled and unidirectional flow is able to reduce the time to reach similar intestinal *in vivo* values from 14 to 4 days compared to those in a non-specific flow.

Therefore, advances in this model will be a useful alternative for obtaining *in vivo* intestinal permeability values in a shorter time compared to 2D cultures (21 days) and will serve as an advanced and improved tool for intestinal studies.

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WHO I AM?

r.lopez@unizar.es

linkedin.com/in/rut-l%C3%B3pez-boix-6a72bb303

https://tmelab.unizar.es/rut-lopez/