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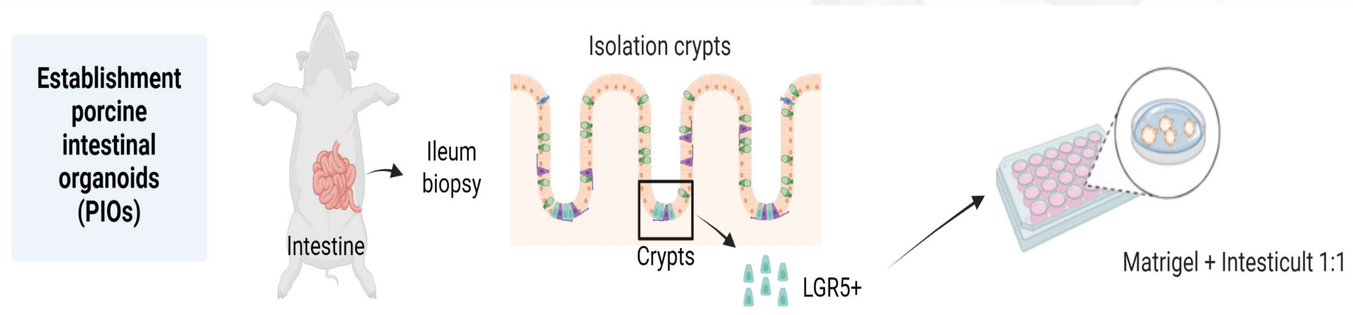
Porcine Organoid-Derived Monolayers in Static and Dynamic Platforms: Toward a Biomimetic *In Vitro* Intestinal Model

Rut López [1,2], Estela Solanas [1]
[1] Tissue Microenvironment (TME) Lab. University of Zaragoza
[2] Instituto de Investigación Sanitaria Aragón (IIS Aragón)

INTRODUCTION

Weaning induces significant intestinal stress in piglets, affecting epithelial proliferation, metabolism, and tissue organization. While 2D monolayers and Transwell systems are widely used *in vitro*, they fail to replicate the structural and functional complexity of the native intestine. Porcine intestinal organoids (PIOs) offer a more biomimetic alternative, reproducing the 3D architecture and cellular diversity of intestinal tissue. However, their static nature and the close configuration limits their utility for functional studies such as absorption or host–microbiota interactions. Organ-on-chip (OOC) platforms address these limitations by providing dynamic, physiologically relevant conditions. This study compares PIO-derived monolayers cultured under static (Transwell) and dynamic (OOC) conditions to evaluate epithelial organization, barrier function, and differentiation in a post-weaning in vitro model.

METHODOLOGY



RESULTS

Establishment of Porcine Intestinal Organoids (PIOs)

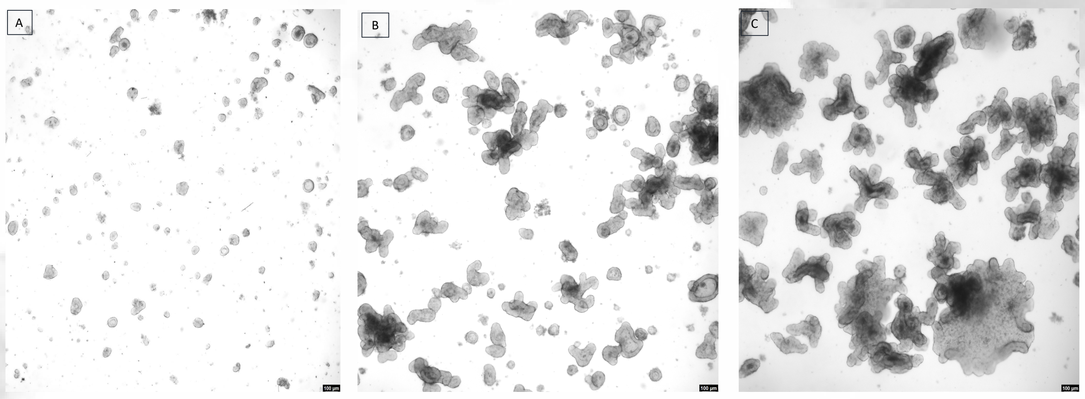


Figure 1. Microscopy images of long-term expansion of porcine intestinal organoids (Passage 4). (A) Morphology of intestinal crypts at day 0; (B) Organoid culture after 4 days; (C) Mature organoids after 7 days in culture. Scale bar = 100 µm. Images 5X.

Representative Expression of Epithelial Markers in Intestinal Organoids

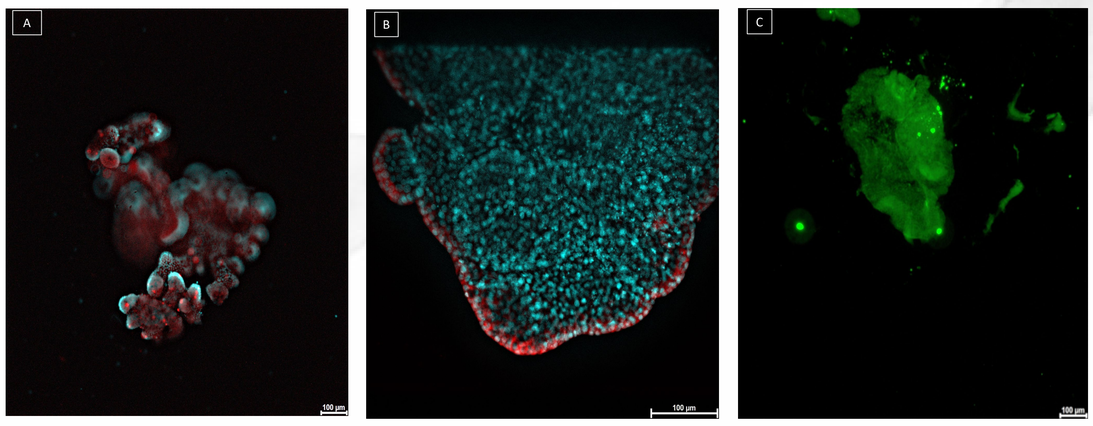


Figure 2. Microscopy of mature porcine intestinal organoids. (A) Tight junctions (ZO-1, red) and nuclei (DAPI) 10X; (B) Microvilli (Villin, red) and nuclei (DAPI) 25X; (C) Adherent unions (E-cadherin, green) 10X . Scale bar = 100 µm.

Barrier Function and Enzymatic Activity in Static vs Dynamic Cultures

Permeability **Alkaline Phosphatase**

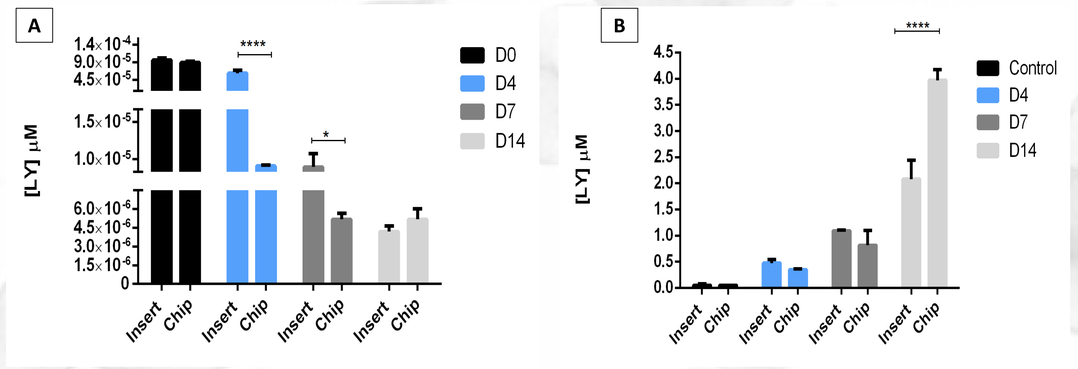


Figure 4. Figure A: Permeability assay using Lucifer Yellow ([LY]) in Insert and Chip platforms over time (D0, D4, D7, D14). Permeability was significantly reduced in the Chip compared to Insert at days 4 and 7; Figure B: Alkaline phosphatase (ALP) activity increased over time, with significantly higher levels observed in the Chip at day 14 compared to all other conditions. * p ≤ 0.05; *** p ≤0.001.

Unidirectional flow accelerates intestinal barrier formation and enhances enterocyte differentiation, making the chip model a more physiologically relevant alternative to static cultures with marker profiles comparable to in vivo

Epithelial Characterization of Monolayers in Static vs Dynamic Cultures

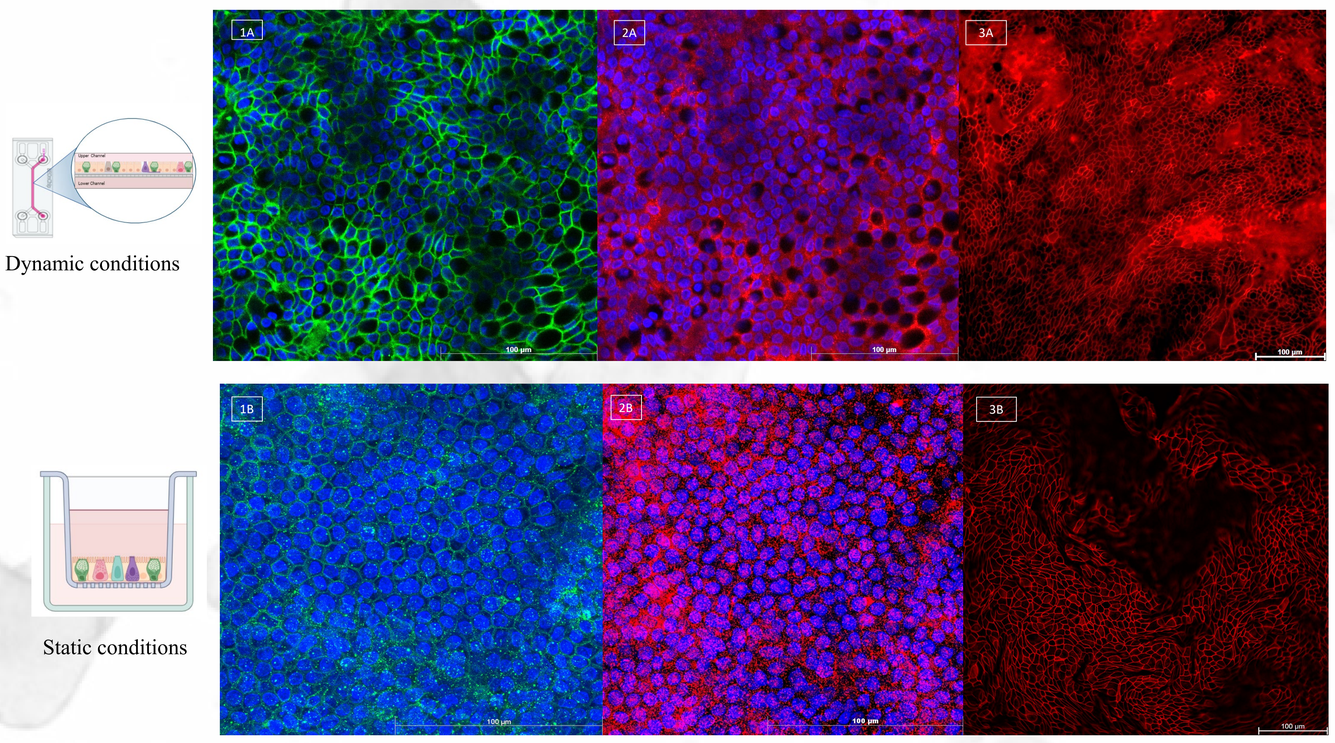


Figure 3. Confocal and Thunder microscopy of the organoid monolayer in Be-Doubleflow and Transwell systems at 14 days. (1A-1B) Adherent unions (E-cadherin, green) and nuclei (DAPI) 63X; (2A-2B)) Microvilli (Villin, red) and nuclei (DAPI) 63X; (3A-3B) Tight junctions (ZO-1, red) 25X. Scale bar = 100 µm.

Static and dynamic monolayer cultures express ZO-1, E-cadherin, and villin. The dynamic chip model shows increased E-cadherin and villin levels, indicating enhanced epithelial differentiation.

CONCLUSIONS

Porcine intestinal organoid-derived monolayers were successfully established under both static and dynamic conditions. The dynamic microfluidic system promoted earlier barrier maturation and enhanced epithelial differentiation, supporting its value as a more efficient and biomimetic platform for modelling post-weaning intestinal physiology *in vitro*.

AKNOWLEDGEMENTS

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