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

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Abstract

Porcine intestinal organoid-derived monolayers were cultured under static and dynamic conditions. The dynamic system promoted earlier barrier maturation, enhanced epithelial differentiation, and stable permeability, reflecting improved physiological relevance. These findings support organ-on-chip platforms as effective, biomimetic tools for studying post-weaning intestinal function *in vitro*, reducing reliance on animal models.

Introduction

Weaning is a critical phase in piglet development, during which animals undergo significant physiological stress that disrupts intestinal epithelial cell proliferation, energy metabolism, and cellular organization [1]. The development of physiologically relevant *in vitro* models has become essential in intestinal research.

Although conventional two-dimensional (2D) monolayers of porcine intestinal epithelial cells are commonly used *in vitro* due to their ease of use, they do not replicate the structural and functional complexity of the native intestine [2]. Transwell systems, with a porous membrane on which the intestinal epithelium lies, offer better polarization, but still do not recapitulate the full architecture and dynamic conditions of the native intestine [3].

Recent advances have enabled the generation of porcine intestinal organoids (PIOs) from intestinal stem cells. These three-dimensional (3D) self-organized structures reproduce the cellular diversity, architecture, and polarization of the native epithelium, and have emerged as a valuable tool for studying innate immune responses and the effect of dietary compounds, particularly in the context of post-weaning intestinal health [4,1]. However, their translational potential for studies involving host–microbiota interactions or compound absorption is limited by restricted access to the lumen and lack of dynamic stimulation, unless complex procedures such as microinjection are employed. In this regard, recently developed organ-on-chip technologies may provide a solution to these limitations. Microfluidic platforms simulate a dynamic, miniaturized environment that more closely replicates native intestinal physiology [5,6]. This study uses monolayers derived from PIOs to model post-weaning intestinal epithelium *in vitro*. We compare their performance in static (Transwell) and dynamic (OOC) platforms to evaluate the impact of microfluidic flow on epithelial morphology, barrier integrity, and function, to advance the development of more biomimetic and translational intestinal models.

Materials & Methods

Intestinal organoids were developed by isolating intestinal crypts from the ileum of post-weaning piglets following the protocol described by Barnett et al. [7]. Crypts were embedded in Matrigel and cultured in a commercial medium (IntestiCult™, STEMCELL Technologies) supplemented with 100 U/mL penicillin, 100 µg/mL streptomycin, 10 mM Y-27632, and 2 mM CHIR99021. After expansion, organoids were cryopreserved until use.

To form monolayers, the organoids were dissociated into small fragments and seeded into a microfluidic device (BE-Doubleflow, Beonchip) under dynamic conditions using a rocker platform. In parallel, static monolayers were established using standard Transwell inserts (1µm pore size). The chips and the static cultures were maintained for 14 days.

Intestinal barrier integrity was assessed by paracellular permeability to Lucifer Yellow, following the method described by Pires et al. [8]. Measurements were taken on days 0, 7, and 14.

Epithelial phenotypes were analyzed by immunofluorescence. Monolayers were fixed in 4% paraformaldehyde for 30–45 min., permeabilized

with 0.1% Triton X-100 for 20 min., and stained with primary antibodies against Villin, ZO-1 and E-cadherin. Nucleus staining was performed with DAPI (1:100) for 1 hour. To assess epithelial organization and marker expression, samples were imaged using a confocal fluorescence microscope.

Results & Discussion

Epithelial monolayers derived from porcine organoids self-organized under both static and dynamic conditions, showing phenotypes consistent with the intestinal mucosa. In the dynamic (chip) model, earlier adhesion and a higher ordered epithelial arrangement were observed from the initial days, suggesting enhanced tissue organization.

Immunofluorescence analysis confirmed the presence of ZO-1, E-cadherin, and Villin in both models, indicating the formation of tight junctions, adherents' junctions, and apical brush borders, respectively. Expression of all markers was consistently lower in static cultures, suggesting reduced epithelial polarization and limited differentiation in this model.

The permeability assay revealed different maturation rates. In the Transwell static model, the epithelial barrier exhibited a gradual reduction in permeability over time ($p < 0.0001$), with statistically significant differences compared to D0. Barrier function continued to improve until D7 ($p < 0.0001$) and remained stable through D14 ($p = 0.33$), indicating a delayed but sustained barrier maturation under static conditions. In contrast, the OoC model showed a rapid decline in permeability as early as D4 ($p < 0.001$), with no further significant changes between D4, D7, and D14 ($p > 0.05$). This indicates that barrier maturation occurs earlier and remains stable over time under flow conditions. This supports the role of mechanical stimulation in promoting robust epithelial differentiation and organization [5,6].

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*.

Acknowledgements

This work was supported by the CDTI project 2022/0297 through Cuarte S.L. Chips were provided by Beonchip S.L. Rut López was supported by an Investigo contract via IIS Aragón.

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