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

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Abstract

This study explores the development of *in vitro* intestinal models on chip using cocultures of Caco-2 and HT29-MTX cells over time under different flow conditions. For this purpose, cocultures in the chip were connected to a syringe pump with a characteristic shear stress of the intestinal epithelium, 0.02dyn/cm2 for 4 days and, compared to cocultures with non-specific flow by rocker for 21 days. The effect of different flow conditions in the intestinal permeability was assessed following the Lucifer Yellow assay. Coculture under specific flow conditions showed similar permeability values after 4 days to those under non-specific flow conditions after 14 days. Therefore, optimalization and further characterization of this model, may lead to a promising alternative for intestinal *in vitro* studies.

Introduction

The gastrointestinal tract is a crucial part of the body for growth and development, and its dysregulation can cause important and multiple diseases. However, research in this field faces significant challenges mainly due to ethical and technical limitations associated with human experimentation and the use of animal models. Therefore, for the study of this main organ, and its pathophysiology, is fundamental the use of reproducible and realistic intestinal *in vitro* models [1].

To mimic the complexity of intestinal epithelium, two-dimensional (2D) and three-dimensional (3D) *in vitro* intestinal models containing enterocytes (Caco-2 cells) and mucus-producing goblet cells (HT29-MTX cells), have been developed to form a differentiated and confluent cell monolayer capable of simulating the barrier between the intestinal lumen and the systemic circulation [2].

To improve more dynamic and realistic culture conditions, microfluidic systems and organ-on-a-chip devices have been developed. These systems allow the study of the intestinal barrier and nutrient absorption [3]. However, this technology needs to be improved to include continuous fluid flows that can promote cell differentiation and better mimic the physiological conditions of the intestine and the cellular response to different shear stress. For these reasons, our study focuses on the development of a model to study intestinal permeability under different flow conditions over time in a microfluidic device to improve the accuracy of *in vitro* intestinal models.

Materials & Methods

Human colon adenocarcinoma cell lines Caco2 and HT29 modified with methotrexate to produce mucus (HT29-MTX) were used in this model. Cells were grown separately and mixed before seeding in a physiologically relevant ratio to represent the small intestine (9:1 Caco-2/HT29-MTX). Co-cultures were seeded on commercial microfluidic devices (BE-Doubleflow chips, BeOnChip) at a density of 5×10^6 cells/cm^2, to form a monolayer on top of the membrane separating the two channels of the chips at day 0. The co-culture was maintained in Dulbecco's Modified Eagle Medium (DMEM) with 10 % heat inactivated fetal bovine serum, 2 mM l-glutamine, 1 % non-essential amino acids, 100 U/ml penicillin and, 100 µg/ml streptomycin, at 37 °C in 5 % CO_2. The chips were maintained in two different flow conditions: under a bidirectional and non-specific flow using a rocker for 21 days, changing the culture medium every 2–3 days (both in the apical and basolateral compartments) or with a controlled and unidirectional flow using a pumping system (syringe pump, Figure 1) with a shear stress of 0.02 dyn/cm² for 4 days at 37°C.

The epithelium permeability was assessed by the Lucifer Yellow method described by Pires et al. [4]. Permeability was measured on days 0, 7, 14 and 21 for chips in rocker and on day 0 and 4 for those connected to the pumping system.

Results & Discussion

Chips under non-specific and bidirectional flow showed a statistically significant decrease in permeability from day 14 in culture compared to the initial monolayer. This permeability continued decreasing over time to the minimum after 21 days in culture ($p<0.001$) (Figure 2, A), reaching values typical of the intestinal mucosa in vivo.

Coculture with controlled and unidirectional flow, under a shear stress of 0.02dyn/cm, showed similar permeability values after 4 days compared to coculture under non-specific conditions by rocker in 14 days (Figure 2, B).

Comparison between both models revealed that the incorporation of a controlled and unidirectional flow reduced the culture time needed to recreate in vivo intestinal permeability values. So, it is expected that specific and unidirectional flow upregulates epithelium differentiation, enhancing intercellular junctions.

Conclusions

Our study shows that coculture of caco-2 and HT29-MTX in chips under controlled and unidirectional flow, is able to reduce intestinal permeability 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 values of the in vivo intestinal permeability in less than 21 days and serves as an advanced and improved model for intestinal studies.

Acknowledgements

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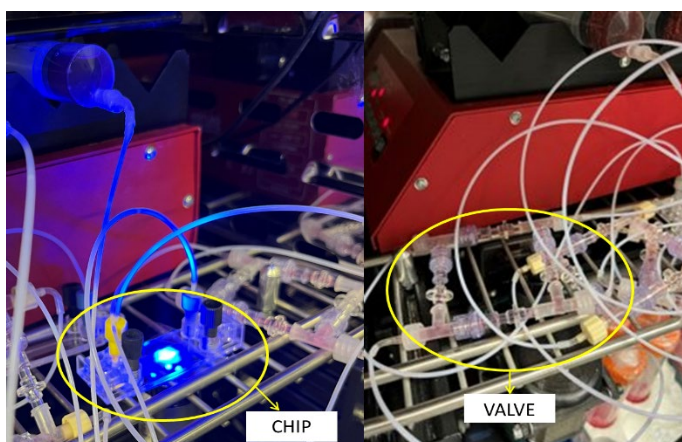

Figure 1. System design of our intestine-on-a-chip model attached to a syringe pump with valve.

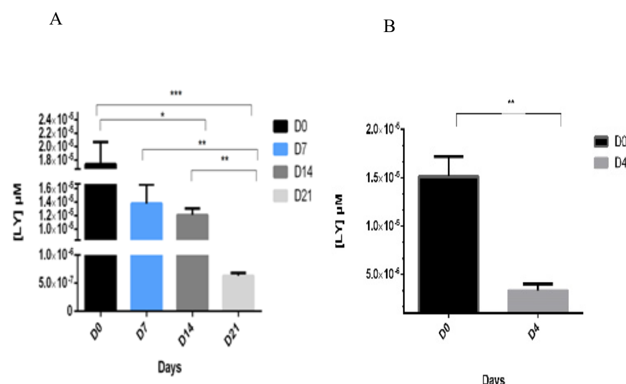

Figure 2. Permeability of coculture in a microfluidic device. A) At day 0, 7, 14 and 21 with non-specific flow. B) At day 0 and 4 with syringe pump. Error bars SEM. (* $p<0.001$, ** $p<0.01$, * $p<0.05$)**