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Trans-epithelial Electrical Resistance (TEER) Measurements on an Artificial Gastrointestinal Tract on a Microfluidic Chip

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Introduction

The concept of nutrikinetics in nutrition research is still in its infancy. To trace fate of nutrients, an artificial gastrointestinal tract, coined the Nutrichip[1], has the potential to significantly improve nutrikinetic measurements. This chip incorporates a confluent monolayer of Caco2 cells, which is a widely used cell type that is used in an *in vitro* model of the gastrointestinal tract to predict the transport of nutrients and stimuli and is mostly studied in a classical Transwell system.

Chip design and fabrication

The chip comprises two PDMS layers sandwiching a porous membrane (Figure 1). Before, we have made chips in which the membrane was treated with air plasma and a silane to form a sealed structure, but toxicity of the silane to Caco2 cells required thorough rinsing and there was a leakage risk during long-term culture. To eliminate the above defects, a clamping approach (Figure 2A) is adapted here for the chip integration. The TEER measurement is a four-point resistance measurement. Four Ag/AgCl electrodes were inserted into the chip in contact with the media and connected to a TEER meter (EVOMX, WPI) (Figure 2B and 3). The confluence of the Caco2 monolayer can be determined by an increase in TEER, and the former is a prerequisite for transport studies of nutrients and/or stimuli through the cell layer.

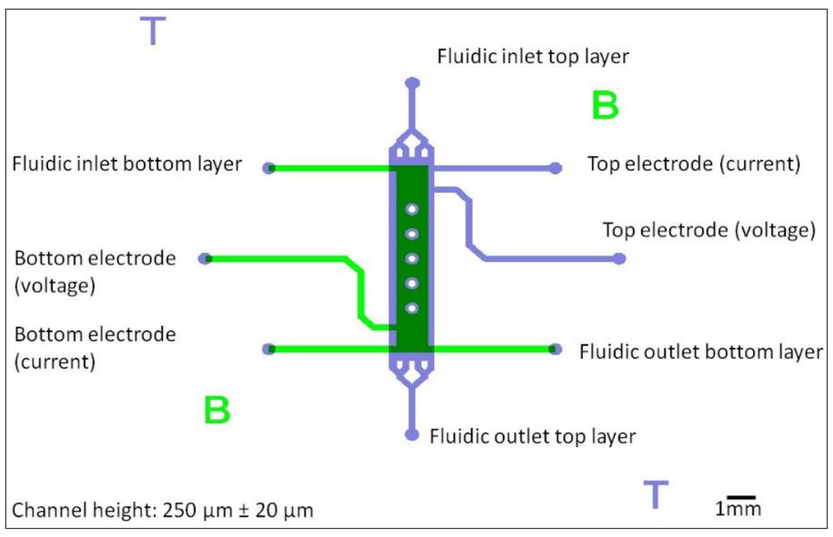

Figure 1: Schematic representation of the two-layer mask design (layers B(ottom) and T(op)) of a microfluidic chip for TEER measurements. To avoid membrane collapse, five PDMS posts are used in the center of the chamber as membrane support.

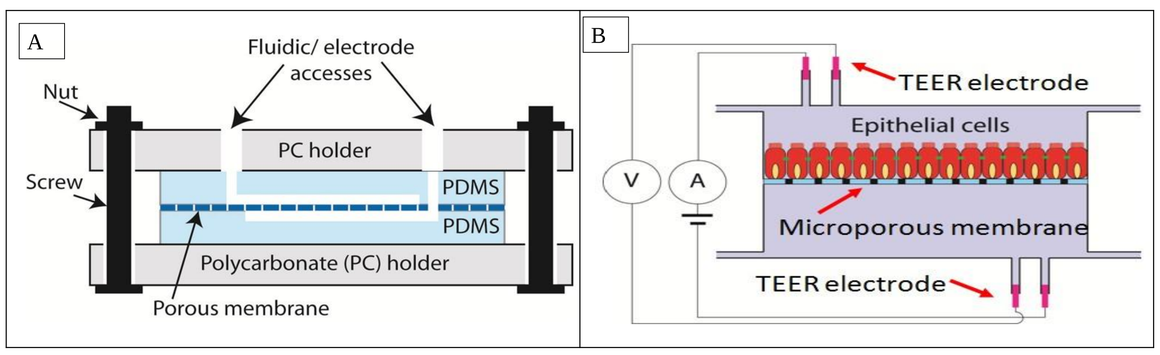

Figure 2. Cross-sectional schematic representation of the clamping holder for the microfluidic chip (A) with indication of the 4 TEER electrodes (B).

Experimental Results

Caco2 culture was first performed in a Transwell device [2], where a confluent layer was formed after 6 days of culture (Figure 4A). A confluent Caco2 cell layer was also obtained after 6 days of culture on chip (Figure 4B). The Caco2 cells were strongly adhering on the porous membrane and resisted to perfusion flow up to 5 nL/s.

TEER of a Caco2 monolayer in the Transwell system was recorded daily. Moreover, Caco2 cells were seeded in the chip and continuously perfused with DMEM cell medium at 5 nL/s. The results in Figure 5 revealed that cells grown increased their TEER over the first 6 days after seeding and then maintained similar high levels for several days of culture both in the Transwell device and on the chip. However, the TEER value of the Caco2 monolayer grown on the chip (Figure 5A) was around 7-fold higher than that in the Transwell system (Figure 5B).

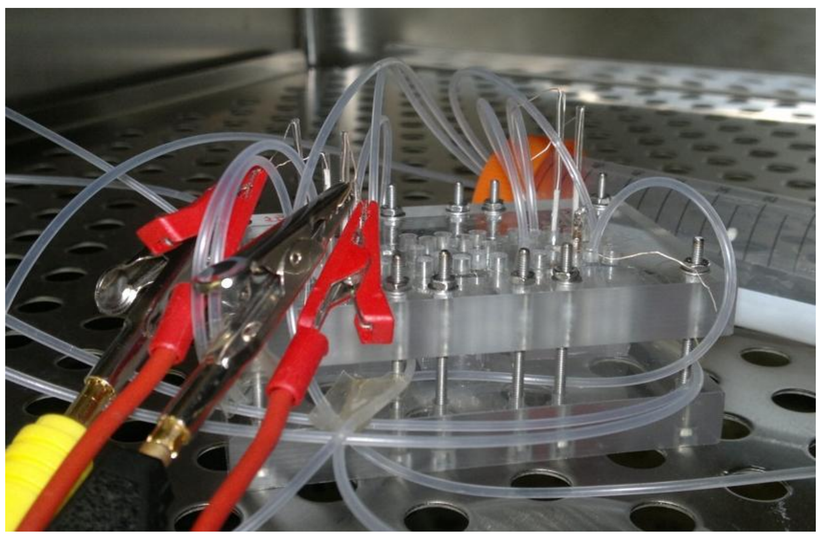

Figure 3. Photograph of a clamped microfluidic chip with connectors for the TEER measurements.

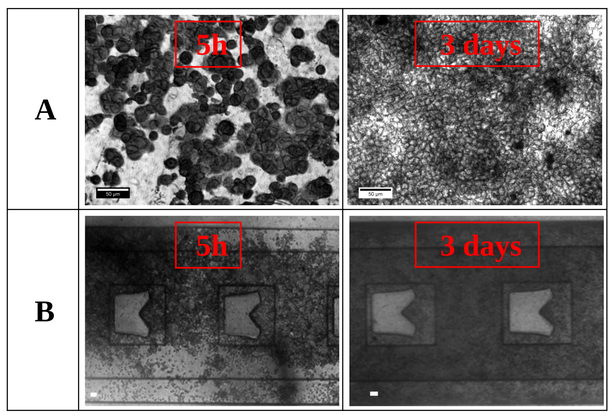

Figure 4. Transmission optical images of Caco2 cells grown in a Transwell device (A) and on the microfluidic chip (B) for different culture times. Scale bar is 50 µm.

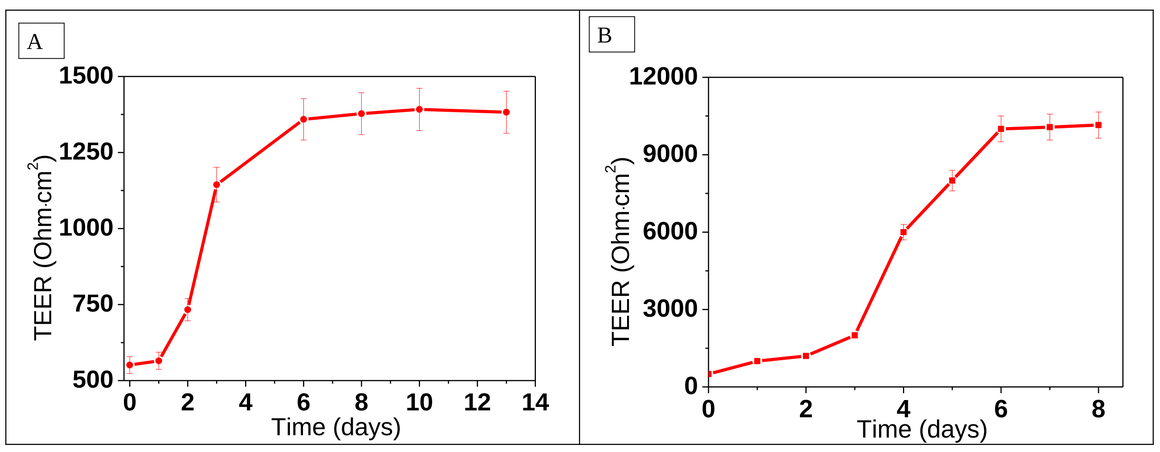

Figure 5. Time dependence of the TEER of a Caco2 cell monolayer cultured in a Transwell device (A) and on the microfluidic chip (B).

Summary and conclusion

In summary, we proposed a microfluidic model that mimicked the gastrointestinal tract. In our study, Caco2 culture has been performed and TEER results confirmed that a confluent monolayer was obtained after 6 days of culture both in the Transwell device and on the chip. In future, this *in vitro* model will be employed to quantify at the same time the TEER and the transport of calcium present in dairy products by using 'wavelength ratio' imaging techniques [3].

References

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2. "The use of Caco-2 cells as an in vitro method to study bioavailability of iron" Maznah I Jr. Malaysian Journal of Nutrition. **5**: 31-45 (1999).
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