Human Organ-on-a-Chip: Around the Intestine Bends

Lucia Giampetruzzi, Amilcare Barca, Chiara De Pascali, Simonetta Capone, Tiziano Verri, Pietro Siciliano, Flavio Casino and Luca Francioso

Abstract The small intestine is the central component of the gastrointestinal (GI) tract (gut) where nutrients are absorbed into the body. Its functional structure is mainly based on its extremely extended surface area, further increased by a specific carpet of villi, responsible for the translocation of nutrients from the GI lumen into the bloodstream. Also, in the small intestine, the absorption processes of the orally administered drugs are basically related to the pharmacokinetics [1]. The deficit of cell culture methods to maintain in vivo–like functions forces researchers to optimize and apply methods in which cells are seeded and cultured under controlled and dynamic fluid flow [2]. Moreover, the lack of predictive human organ models has increased the necessity of approaches for proper mimicking of organ function in vitro, studying physiological parameters that regard mechanical, chemical and physical stimuli crucial for differentiation, morphology and function of the epithelia [3]. In this work we present a Gut-On-Chip (GOC) device, equipped with ITO (Indium tin Oxide) electrodes patterned by wet etching techniques, as a multifunctional microsystem for monitoring epithelial parameters. The potential to support cells adhesion, growth and polarization of a functional monolayer is also investigated in the Caco-2 epithelial-like cell line by in-device seeding and culture. In a perspective, this first prototype has established the basis for several technology integrations to study complex cellular phenomena targeted in key physiological topics (e.g. the tight interplay of different physical effects during mechanotransduction processes) and in pharmacological open issues such as drug absorption and metabolism.

Keywords Gut-On-Chip (GOC) · Organ-On-Chip (OOC) · TEER Mechanical stimuli · Epithelial-like behavior · Embedded sensors

L. Giampetruzzi (✉) · C. De Pascali · S. Capone · P. Siciliano · F. Casino · L. Francioso
Institute for Microelectronics and Microsystems IMM-CNR, Via per Monteroni "Campus Ecotekne", 73100 Lecce, Italy
e-mail: lucia.giampetruzzi@le.imm.cnr.it

A. Barca · T. Verri
Department of Biological and Environmental Sciences and Technologies (DiSTeBA), University of Salento, Via per Monteroni "Campus Ecotekne", 73100 Lecce, Italy

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1 Introduction

The gastrointestinal (GI) tract shows extreme peculiarities at each (from micro to macro) scale of structural-functional organization, due to its complex interplay of residing cells, tissues and organs. The small intestine is one of the most important component of the digestive system. It is a considerably extended tube combined with the occurrence of peristaltic movements that are essential for solute and water movements and/or elimination of waste products. The surface area of this "small" tract is significantly enhanced, 30–600 fold, respectively, by the carpet of finger-like projections called intestinal villi, as well as the microvilli, cellular membrane protrusions present on enterocytes (i.e. differentiated epithelial cells).

Villi and microvilli are structural features specialized both for nutrient/ion/water absorption and secretion, and for mechanotransduction. In this scenario, epithelial tight junctions (TJs) are key elements in maintaining the epithelial barrier function and in regulating the permeability of nano-/micro-/macromolecules. They are present in between the epithelial cells keeping the luminal content from leaking between cells, so almost all nutrients go through the gut epithelium to the circulatory system in a strictly controlled manner.

The importance of the small intestine epithelium along the GI tract also concerns the absorption of orally administered drugs into blood across the highly polarized epithelial cell layer and the intestinal mucosa. In this respect, also the peristaltic movements and the intestinal microbiota are primary modulators of the bioavailability of the orally administered drugs [4]. This suggests that there are many intertwined mechanisms that affect the therapeutic efficacy of drugs, especially in certain pathological conditions of the GI, which comprehensively contribute to increase the difficulty in reproducing accurately the cyto/histological features of the simple columnar epithelium and the principal physiological processes occurring in small intestine [5].

To date the static planar culture models (in vitro, or ex vivo) and ethics issues related to in vivo assays do not allow to take into account more than a few crucial functions of the intestinal tracts. Moreover, due the peculiar geometry of intestinal epithelium (i.e. corrugation, convolution), the fluid dynamics are missed in the static two-dimensional (2D) in vitro models, which often fail to reproduce the physiology of e.g. drug metabolism, drug absorption, drug–drug interaction, etc. [6].

Over time, this failure of reproducing several living organ aspects has fueled the development of many organs on-a-chip approaches (Fig. 1).

Recently, microfabrication techniques have been used to replicate key functional units of small intestine as: unique topography of intestinal epithelium and the 3D cell physiology of intestinal crypts.

These micro-devices, based on micro-engineered biomimetic systems containing microfluidic channels, human cell-lined in order to simulate intestinal epithelia, in its properties, eventually combined with microbial symbionts.

The accurate reproduction of the in vivo environments, with controlled microfluidics parameters helps to reduce the delays and costs of research and to replace animal tests.

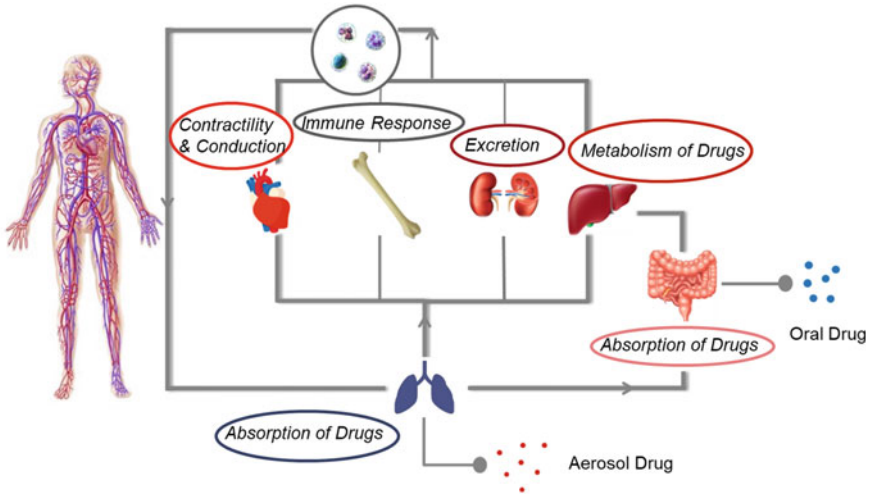

Fig. 1 The Human on a chip concept inspired form Wyss Institute (https://cdn.thenewstack.io/media/2015/04/organs-on-a-chipwyss-institute-3.jpg)

The technology is mainly based on a microfluidic technique, which enables manipulating small amount of liquids, controlling fluid flows in order to deliver nutrients to the biological matrices. The microfluidics is supported by microfabrication that creates microstructures able to control cell shape and function [3].

Considering recent studies of an intestine on a chip, some models were adapted from e.g. lung and heart to mimic the mechanical/structural properties, better reproducing the in vivo environments, but presenting some disadvantages and open issues, yet [7].

The principle limits are the inaccurate simulation of the intestine's peristalsis movements (inducing membrane curving, stretching, bending), the little standardization among companies and research groups, the lack of extensive analyses and considerations on cell culture procedures (cell seeding and attachment, growth, effects of nutrients and stimuli) and the in situ sensing network still lacking [7, 8].

In the perspective of developing a multifunctional Gut-On-Chip (GOC) device, we hereby propose a GOC with an integrated detection of monitoring in real-time several parameters. The transcellular and paracellular transport processes are important issues to investigate the tightness and the mechanisms of absorption and diffusion; similarly, the role of membrane-trafficking/exchange and nutrient/drug fluxes remain underappreciated and unexplored. The device is equipped with micromechanical stimulation sensors that can apply physiologically relevant mechanical stimuli and with electrodes able to control the pH gradients, the plasma membrane potentials and solute gradients as metal ion uptake or translocation. This approach presents many advantages, mainly related to the integrated detection possibilities and multiparameter investigation (Fig. 2).

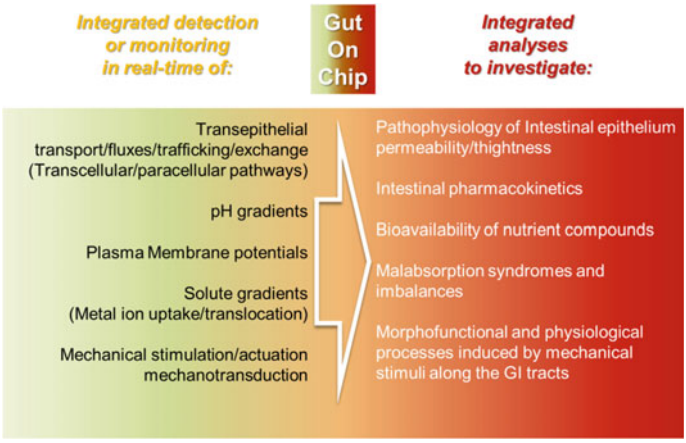

Fig. 2 Potential integrated analyses enabled by Gut-On-Chip devices

Although this study cannot cover all parameters of the gut physiology, by implementing a scheme of an integrated gut on-a-chip it hints the intention of elaborating some microtools equipment to monitor important cell-interaction device parameters, less investigated in microfludic platforms; where they appear more critical and unpredictable than in traditional in vitro cell culture. It also aims to overcome the limits of the novel technology designing a device able to achieve the recent pharmaceutical and medical challenge.

2 Materials and Methods

The GOC platform is composed of a customized commercial Topas polymer-made base with two (lumen vs. blood) fluidic chambers separated by a PET (Polyethylene terephthalate) porous membrane for cell culture (23 μm thickness, 200 nm pore dimension, $5 \cdot 10^8$ pores/cm^2 pore density) (Fig. 3). Membrane size is 11×8.5 mm^2. The fluidic platform is equipped with transparent ITO (Indium tin Oxide) electrodes for TEER monitoring during the cell culture; in fact, that TEER (Transepithelial Electrical Resistance) values are worth to measure integrity of cellular barriers and tight junction dynamics of cultured cells monolayers.

Our re-sealable assembly allows start cell culture with the porous membrane exposed to medium with no cover lid; once cell culture is stabilized, the chip chamber is sealed on top and bottom sides with Corning or Topas slides equipped with 200 nm thick ITO electrodes patterned by wet etching technique. So, 2 independent fluidic cham-bers, apical and basolateral, with a capacity of 50 μl per chamber were created. Major advantage of our device is that it allows software-controlled mechanical deflection of the membrane suspended between the two fluidic chambers, both

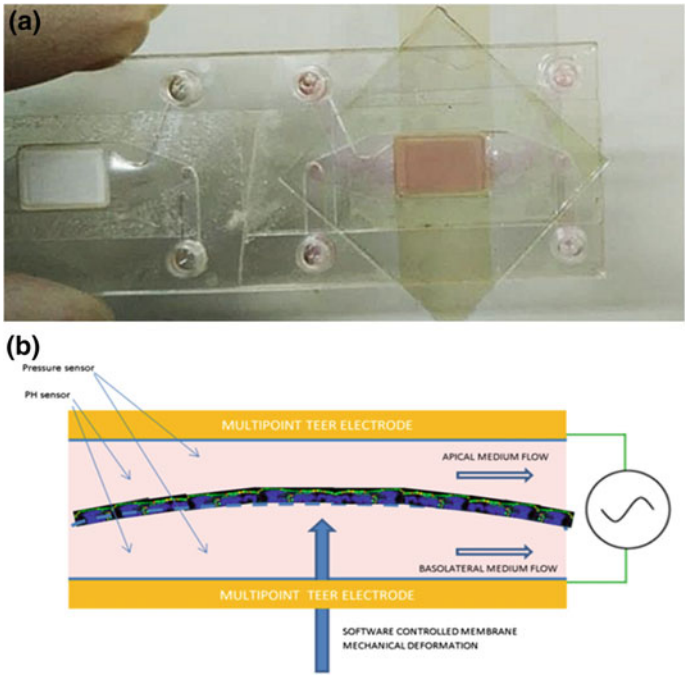

Fig. 3 Modified commercial multifunctional platform for Gut-On-Chip Caco-2 cultures with embedded ITO electrodes (**a**) and schematic of chip concept for multisensorial capabilities (**b**)

above and below the membrane, and simultaneous embedded sensors measurements; which helps in reproducing local mechanical stretches and bends, comparable to an in vivo situation, and monitoring effects in continuum.

We selected Caco-2 cells (human colorectal adenarcinoma-derived) as cellular model [9], suitable to preliminarily investigate cell proliferation and monolayer-to-chip interactions on the PET porous membrane.

The adhesion of Caco-2 cells on chip membrane was evaluated seeding at a density of about 2.5×10^4 cells/cm^2. Then, a flux to completely refresh the medium in the chamber every 10 min was activated and sustained for 24 h post seeding at 37 °C. After 24 h culture, the presence of viable cells was identified by metabolization of the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] compound.

3 Results and Discussions

Considering the importance of creating a biomimetic chip device to expose cells to a microfluidic flow, we basically evaluated the cell interaction with a PET translucent porous membrane (a non-biological matrix), investigating the cell behavior on an

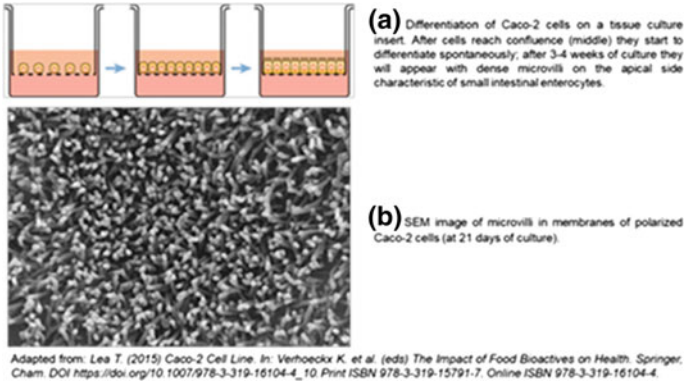

Fig. 4 The Caco-2 enterocyte-like model

early GOC prototype in order to improve the weaknesses and the critical aspects de-riving from biological components. For example, the maintenance of sterility in a dynamic biological system, the viability of cells in a small medium volume for long periods in order to form epithelial barriers and the presence of air bubbles effects on culture are challenging issues deriving from the devices micrometric features and peculiarities. Indeed, the multifunctional microsystem for monitoring epithelia parameters has been set up with proper material and microfluidics tubes and it has primary investigated in its ability to support cell adhesion and growth, for several days, before electrical and mechanical test on the epithelium. Notably, the epithelial-like behavior by spontaneous differentiation of Caco-2 into absorptive enterocytes has been previously demonstrated (Fig. 4).

As Caco-2 cells are able to form a functional monolayer, our cell-to-device combination may represent a useful tool to study: (a) the epithelial tightness and the mecha-notransduction as key tools of the regulatory pathways at cellular and epithelial level in the GI tissue districts; (b) solutes gradients and pH variations across the apical and basolateral chambers for intestinal pharmacokinetics; (c) GI drug toxicity and drug screening.

In our experimental setup, viable Caco-2 cells have been detected by metabolization of the MTT compound producing the dark blue-to-black staining (i.e. formazan crystals in viable cells) (see Fig. 5). Figure 5 shows the centripetal migration front of the growing monolayer of cells, highlighting a growth surface coverage of 80–85%. The MTT assay is a preliminary evaluation to demonstrate a considerable efficiency of adhesion, viability and migration of human-derived intestinal cells on microfluidic devices.

The idea of in vivo mimicking conditions in a more reliable way, provides the optimization of the environmental constraints of on-chip cell cultures and the implementation of some enabling technologies to achieve a real time monitoring of parameters that playing an important role in several physiological pathways.

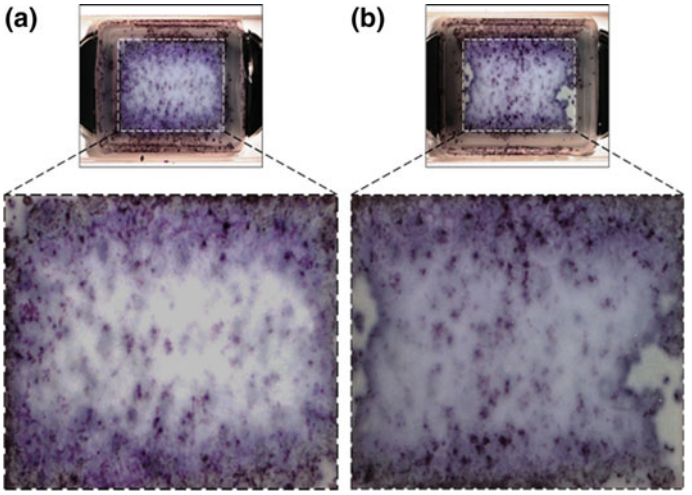

Fig. 5 MTT assay on Caco-2 cells loaded in-device, after cell adhesion and proliferation. Violet dark-blue crystals indicate the presence of viable, proliferating cells in two sample replicates (**a** and **b**)

To measure pH, Zn and Cu passage through the apical and basolateral chamber, miniaturized Ion Selective Electrodes (ISE) will be implemented. Impedance measurements will conduct to allow the detection and evaluation of the metal ions concentrations in situ. To this purpose, the mechanotransduction and sensitivity could represent the keystone to understand important mechanisms to proper reproduce GI tissue and to conduct pharmaceutical tests based on nutrient and drugs intestinal interactions.

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