Intestinal epithelium on chip for absorption studies

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ABSTRACT

Absorption of orally administered therapeutics occurs at the gastrointestinal tract level and its efficiency is paramount for their success through the drug development pipeline.

Current methods used to assess drugs absorption lack in recapitulating 3D physiological microenvironment of intestinal epithelium. Organs-on-chip (OoC) models have been lately proposed to overcome this limitation. We developed an in-vitro model of intestinal epithelial barrier in an OoC able to mimic peristaltic motion by providing a controlled mechanical stimulation. The created barrier functionality was assessed through immunofluorescence analyses and permeability tests.

CONTEXT

Development of new therapeutics is an expensive and risky process: success rate of candidate drugs is lower than 0.1% with severe waste of investments, in terms of money, time and human safety [1].

The main reason for such high failure can be ascribed to the poor efficacy and safety predictive power of preclinical studies. Animal models, despite their intrinsic ability of replicating systemic responses, often fail in faithfully recapitulating human biology due to interspecies mismatch. On the other hand, *in-vitro* human models such as standard 2D cultures, although based on human-derived material, often fail to properly mimic the *in-vivo* biological complexity [2], [3]

Development of accurate human *in-vitro* models is thus crucial to speed up translational processes and better predict the outcome of clinical trials[4].

In the last ten years increasing attention has been dedicated to the **Organs-on-Chip** technology (OoC). OoCs are miniaturized *in-vitro* culture systems able to mimic the *in-vivo* microenvironment by controlling cell-cell and cell-matrix interactions as well as biochemical and mechanical cues [5]. In this scenario, the study of absorption, distribution, metabolism and excretion (ADME) processes is crucial to assess the efficacy of drug candidates. The greatest share of newly developed compounds is orally administered, and their absorption mainly occurs at the gastrointestinal tract level, making the **intestinal epithelium absorption** of paramount relevance for drug candidates' success [6].

Current 2D methods used to assess gut absorption exploit monolayer structures made of gut epithelial immortalised cells (mainly CACO-2) cultured on porous membranes (transwell systems) that are used as a model of intestinal barrier. However, such model lacks in recapitulating the 3D and dynamic microenvironment of intestinal epithelium characterized by crypts and villi structures and peristaltic motion. OoC models have been lately proposed to overcome this limitation [7].

The purpose of this work was to develop an *in-vitro* model of intestinal epithelial barrier in an OoC capable of mechanically stimulate the cultured cells. In this regard, a **mechanical stimulation with sinusoidal pattern** was adopted in order to best replicate the phenomenon of peristalsis and induce the functional maturation of the tissue.

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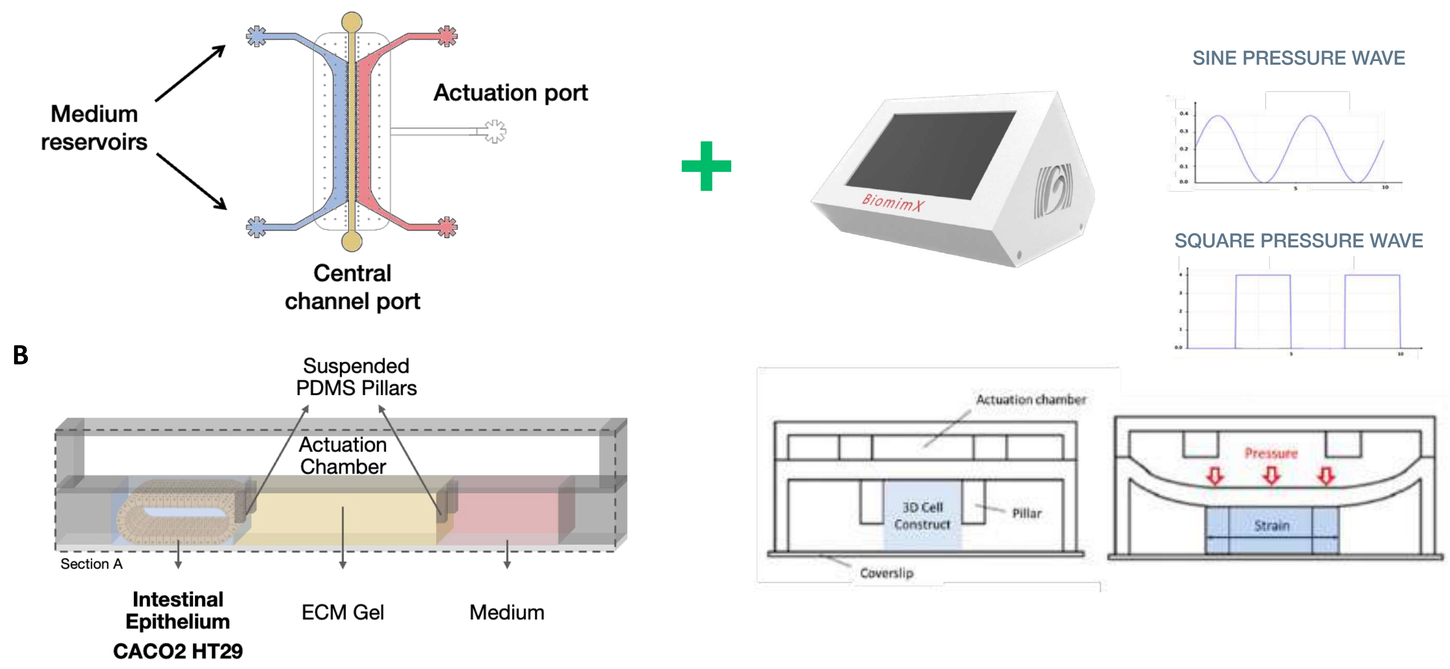
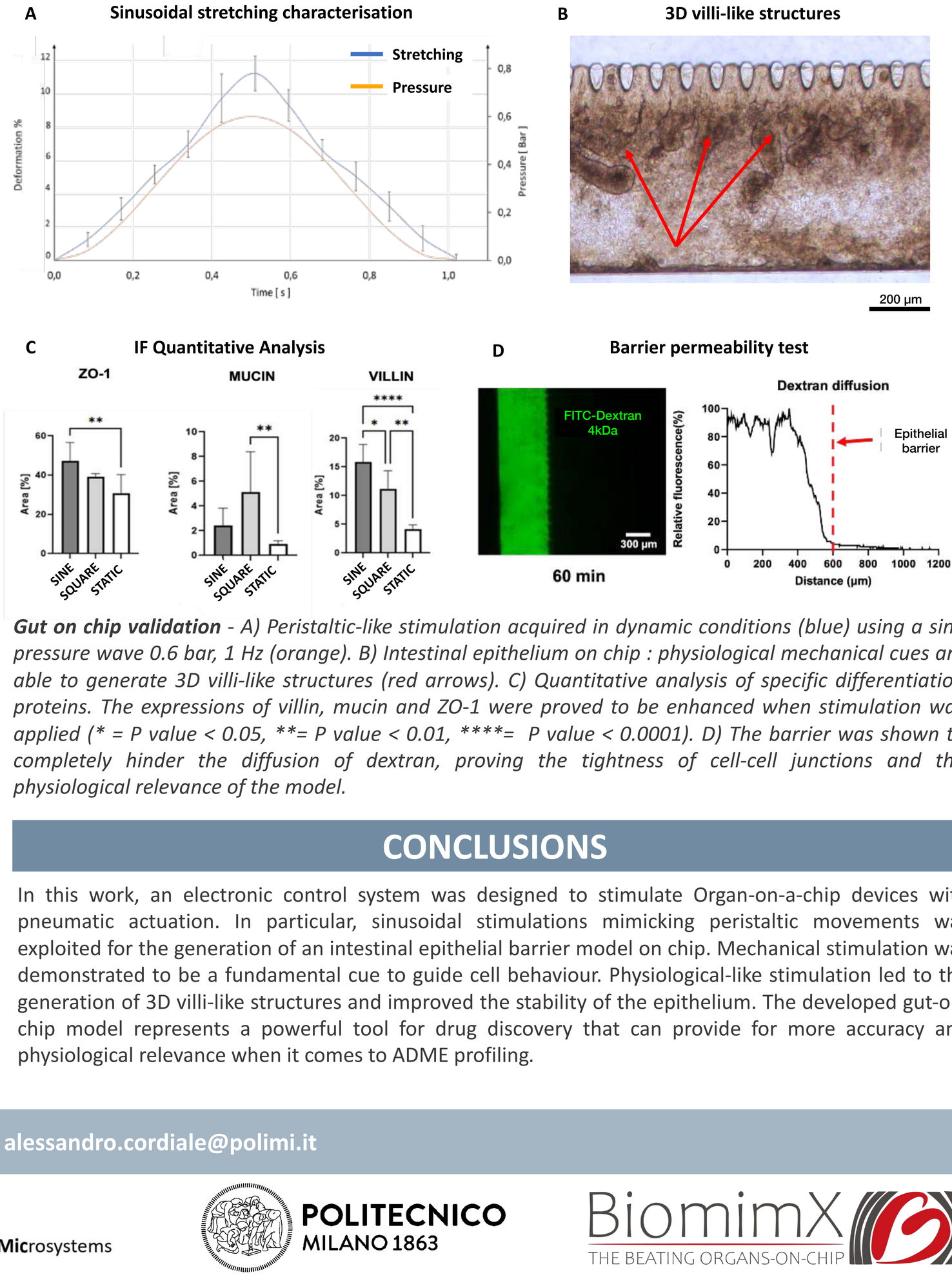
METHODS



Gut on chip model *- A) Top view of the adopted chip showing the ports for medium change and cell seeding, and for ECM (fibrin hydrogel) injection. B) Cross-section of the device showing the biological experiments configuration. C) Stimulation of the gut on chip through an electronic control system: the pressurisation of the pneumatic chamber allows the physiological-like stimulation of the epithelium.*

RESULTS



Gut on chip validation *- A) Peristaltic-like stimulation acquired in dynamic conditions (blue) using a sine pressure wave 0.6 bar, 1 Hz (orange). B) Intestinal epithelium on chip : physiological mechanical cues are able to generate 3D villi-like structures (red arrows). C) Quantitative analysis of specific differentiation proteins. The expressions of villin, mucin and ZO-1 were proved to be enhanced when stimulation was applied (* = P value < 0.05, **= P value < 0.01, ****= P value < 0.0001). D) The barrier was shown to completely hinder the diffusion of dextran, proving the tightness of cell-cell junctions and the physiological relevance of the model.*

CONCLUSIONS

In this work, an electronic control system was designed to stimulate Organ-on-a-chip devices with pneumatic actuation. In particular, sinusoidal stimulations mimicking peristaltic movements was exploited for the generation of an intestinal epithelial barrier model on chip. Mechanical stimulation was demonstrated to be a fundamental cue to guide cell behaviour. Physiological-like stimulation led to the generation of 3D villi-like structures and improved the stability of the epithelium. The developed gut-on-chip model represents a powerful tool for drug discovery that can provide for more accuracy and physiological relevance when it comes to ADME profiling.

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