Development of *in vitro* intestinal barrier model for predictive pre-clinical evaluations

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Intestinal drug absorption represents a pivotal step for drug candidates to enter in clinical trials. However, pre-clinical evaluations are mainly performed in oversimplified conditions enabled by standard 2D culture systems, failing in replicating the physiological environment, or in animal models. Therefore, the translational potential of intestinal *in vitro* models to humans is limited. We developed a novel cartridge-based bicompartmental platform, named True Tissue on Platform (TTOP), which allows to host various culture substrates and to retrieve the biological sample in a controlled manner for endpoint analyses.

The platform was validated with endothelial (EA.hy 926) and intestinal epithelial (Caco-2) cell lines. Comparative studies were carried out to evaluate the effects on the cultures of coatings and substrates, such as gelatin-based and silk fibroin scaffolds, with respect to bare polycarbonate membranes. Co-culture experiments of these cell lines were conducted to replicate the Gut-Vascular Barrier (GVB). To assess barrier functions, Trans Endothelial/Epithelial Electric Resistance (TEER) was measured throughout the cultures. At endpoint, samples were fixed, stained (DAPI, ZO-1) and imaged.

Validation experiments confirmed the suitability of the platform both for endothelial and epithelial models, by assessing proliferation and barrier function (TEER, ZO-1) for up to 21 days. The addition of ECM-like substrates significatively promoted proliferation and differentiation, enabling the formation of 3D cellular constructs after 7 days. An easy co-culture protocol was developed, and good tissue maturation was observed. By hosting different substrates in the same support, we demonstrated, in an unbiased manner, the importance of the extracellular environment in promoting the formation of 3D constructs in static conditions. Preliminary results on co-culture experiments will enable the development of a 3D GVB model to better mimic intestinal drug absorption.

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Conference presentation: this paper was presented at the Fourth Centro 3R Annual Meeting - The role of 3Rs in the age of One Health: where we are and where we're going - 13-15 September 2023, Università degli Studi Milano-Bicocca.

Biomedical Science and Engineering 2023; 4:231
doi:10.4081/bse.2023.231