A comparative approach to recapitulate intestinal physiological absorption *in vitro*, using a novel, modular and versatile MicroPhysiological platform

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The absorption of orally administered drugs through the intestinal barrier is crucial for determining their bioavailability. However, current *in vitro* models have limited reliability, primarily because they are based on static, 2D cultures. To address this issue, we have developed True Tissue On Platform (TTOP), a cartridge-based, modular and versatile *in vitro* platform. Initially, we placed the cartridge in an "open-well" static module and cultured CACO-2 intestinal epithelial cells using standard protocols. We monitored the Trans-Epithelial Electrical Resistance (TEER) during the cultures and obtained differentiated and polarized cell monolayers after 7 days, as confirmed by the expression of Human Epithelial Antigen (HEA) and Junction Adhesion Molecule (JAM). In parallel, we incubated CACO-2 cells at day 12 with or without 100µM Lucifer Yellow (LY) to evaluate cell permeability. Similarly, we integrated and cultured EpiIntestinal™ samples, which are human 3D Small Intestinal Models (SMI) from MatTek™, for 12 days in TTOP static devices. We evaluated and compared TEER, absorption of 10mM caffeine (2h), and LY paracellular passage with MatTek™ controls and CACO-2 data. SMI samples were stained with HEA and DAPI at different time points. Confocal microscopy, made possible by the controlled retrieval of the cartridge, demonstrated preserved 3D villi-like tissue morphology at all time points. The controlled retrieval of the cartridge also allowed us to perform sequential treatments. Specifically, after 7 days of static preparation, CACO-2 cartridges were plugged into a "closed-well" perfusion module, and recirculating flows were applied for 24 hours. The versatility of TTOP enabled us to compare 2D immortalized and 3D primary intestinal cell cultures, thereby reducing inter-device artifacts. Moreover, the introduction of controlled flows will pave the way for more relevant intestinal models, aiming at reducing the need for animal testing in drug absorption studies.

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