Two-dimensional cultured intestinal stem-cell derived organoids as a model for intestinal permeability and transport

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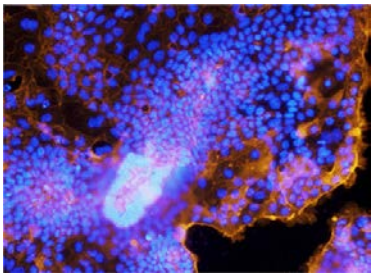
Isolated adult intestinal stem cells have unlimited dividing capacity, produce daughter cells that differentiate into all intestinal cell types and *in vitro* they self-organize into 3-dimensional 'mini-gut'-structures. Compared to epithelial cancer cell lines (*e.g.* Caco-2, HT-29, IPEC-J2) that are routinely used as *in vitro* intestinal models these organoids have major advantages because they are physiological cells without the side-effects of cancer cell lines, all intestinal cell types are present compared to only epithelial cells, they can be obtained from any organism or disease model and they retain intestinal segment specificity.

To test the intestinal effects of nutritional and pharmaceutical compounds, however, these 3D cultured organoids have two main drawbacks: 1) They consist mainly of crypt cells and largely lack epithelial cells, the most common cell *in vivo* and 2) The gut lumen is on the inside making exposure and dosing of compounds challenging.



We developed a 2-dimensional culture system that allows culturing organoids into a confluent intestinal layer that contains both crypt and villus regions, as is the case in the *in vivo* situation. Cells become polarized and, when grown in a transwell format, an upper apical (gut lumen) compartment and a lower basal (body) compartment can be created. Functional assays show a build-up of electrical resistance across the intestinal layer and low leakage of a fluorescent marker, both indicative for an intact intestinal layer.

To further develop and validate this 2D intestinal organoid model more studies are needed. For example, absorption studies using model compounds to show whether *in vitro* derived data in our 2D organoid model correlates with existing absorption data in the animal of human and if this model has a better predictive value than currently used epithelial cancer cell lines.