# A 3-Dimensional Tissue Culture Model for Studying Bile Acid Regulation of Barrier Immunity

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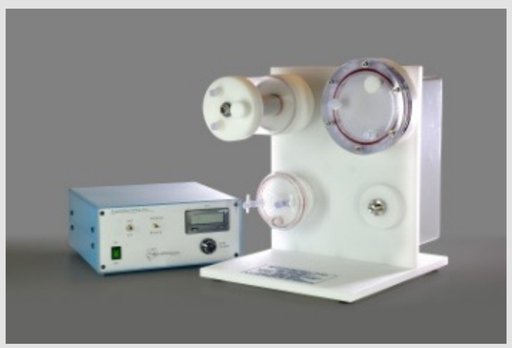
## Introduction

Primary bile acids (BAs) are produced in the liver from cholesterol and are released into the small intestine to aid in the digestion and absorption of dietary lipids. Most of the BA pool is reabsorbed through enterohepatic circulation, but approximately 5% passes into the colon where the intestinal microbiota convert primary to secondary BAs. These secondary BAs directly impact other members of the microbiome and modulate host metabolism and immunity by signaling through host receptors such as the farnesoid X receptor (FXR)[1]. The impact of BAs on barrier immunity in the intestinal epithelium is poorly understood, in part due to a lack of tractable in vitro models. We show that 3-dimensional 'organotypic' cultures express bile acid receptors and immune factors, opening the doors to an experimentally tractable model for studying bile acid impacts on immune functions in the intestinal epithelium[2].

## Methodology

**Growing Organotypic Cultures (OTCs)**

Caco-2 cells (ATCC HTB-37) were cultured two-dimensionally using standard mammalian cell culture protocols. 10 million cells were resuspended in D10 media, mixed with collagen-coated porous dextran beads (Cytodex-3), and used to seed slow turning lateral vessel bioreactors (STLVs) containing 50ml/reactor. After 21 days in continuous culture, the OTCs were harvested and cells from both the 2D and 3D cultures were processed by scRNA-seq using a chromium controller (10X Genomics).



*Synthecon RCCS-4H bioreactor*

**Imaging OTCs**

The OTCs were fixed with 4% paraformaldehyde, permeabilized with triton X-100 and stained using standard immunofluorescent staining protocols. DAPI was used to stain nuclei, UEA-1 was used to stain mucins, and Ezrin was used to stain apical brush borders. The cells were then imaged using a Leica SP5FLIM inverted microscope.

## Results

**3D cultures generate a population of epithelial cells that exhibit phenotypic heterogeneity.**

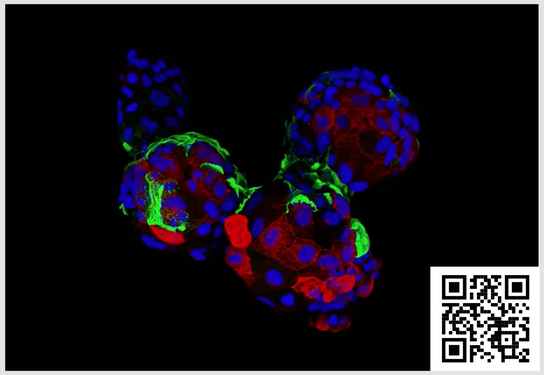


*Figure 1. Immunofluorescent confocal microscopy image of 3D organotypic cultures grown in STLV bioreactors. Nuclei are stained blue (DAPI), mucins are stained green (UEA-1), and apical brush borders are stained red (Ezrin).*

The Ezrin positive staining suggests that cells are polarized on the beads (apical bush border formation). UEA-1 staining may indicate mucous production and possible differentiation into goblet-like cells. An animated version of the imaged 3D cultures can be accessed using the QR code above.

**3D cultures generate unique transcriptional states in Caco-2 epithelial cells.**

UMAP clustering (after cell cycle regression) revealed 8 transcriptionally distinct clusters in 2D and 3D cultures. Clusters 4, 5, 6, and 7 are unique to the three dimensionally cultured cells.

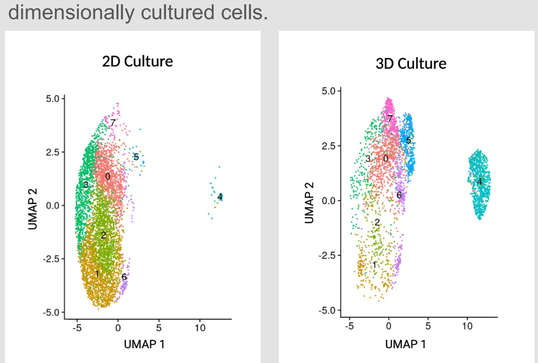



*Figure 2. UMAP clustering of both 2D and 3D cultured cells derived from single cell mRNA seq.*

## Results (cont.)

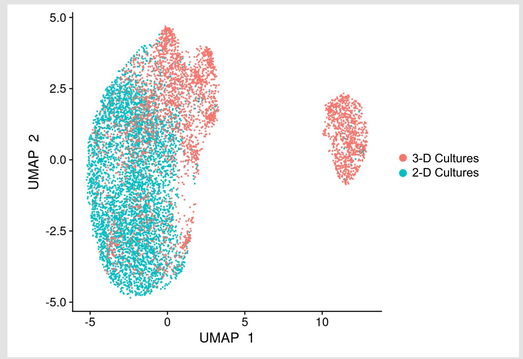



*Figure 3. Overlayed UMAP of 2D and 3D cultures*

**Cell clusters specific to 3-D cultures contain BA receptors and innate immune factors.**

Clusters 4 and 5 are characterized by the expression of interferon genes while cluster 7 is characterized by the expression of bile acid receptor genes.

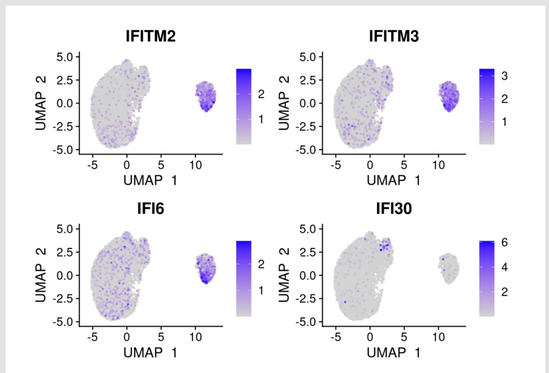



*Figure 4. Interferon-inducible genes highlighted from clusters 4 and 5*

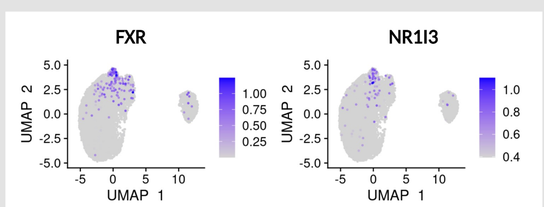



*Figure 5. bile acid receptor genes highlighted from cluster 7*

## Conclusion and Future Directions

Taken together, the imaging and scRNA-seq results suggest that the OTCs grown using STLV bioreactors exhibit a heterogeneity not found in traditional two-dimensional cultures. 3D cultures express higher levels of interferon-inducible innate immune genes and bile acid receptors, compared to their 2D counterparts. This unique transcriptional program, together with the ability to easily dispense 3D bead cultures to 96- or 384-well plates, makes them ideal for use high-throughput screens. We plan to take advantage of these properties in the following future experiments:

Interrogating the impact of BAs on innate immune signaling

3D cultures will be plated in 24-well plates and stimulated for 6hrs with IL23, IL1-beta, IL18, IFN-gamma, or TNF-alpha, in the presence or absence of secondary BA. The cells will then be recovered, and their RNA extracted using a Qiagen kit. This should further inform the role of secondary bile acids in modulating epithelial cell responses to inflammatory signals.

Develop a luciferase read-out for BA signaling in OTCs

Given that our scRNA-seq data show expression of FXR and CAR specifically in 3D cultures, we hypothesize that these cells will respond to secondary BAs added to the media. To test this, 3D cultures will be generated from a FXR-luciferase Caco-2 reporter line (in progress). Our long-term goal is to use this reporter line to screen for microbial metabolites that augment the bile acid signaling.

## References

1 Ikegami, Tadashi, and Akira Honda. "Reciprocal interactions between bile acids and gut microbiota in human liver diseases." *Hepatology Research* 48.1 (2018): 15-27.

2 Drummond, Coyne G., Cheryl A. Nickerson, and Carolyn B. Coyne. "A three-dimensional cell culture model to study enterovirus infection of polarized intestinal epithelial cells." *MSphere* 1.1 (2016): e00030-15.

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