Applications of Human Intestinal Organoids

Lecture 4

Presenter
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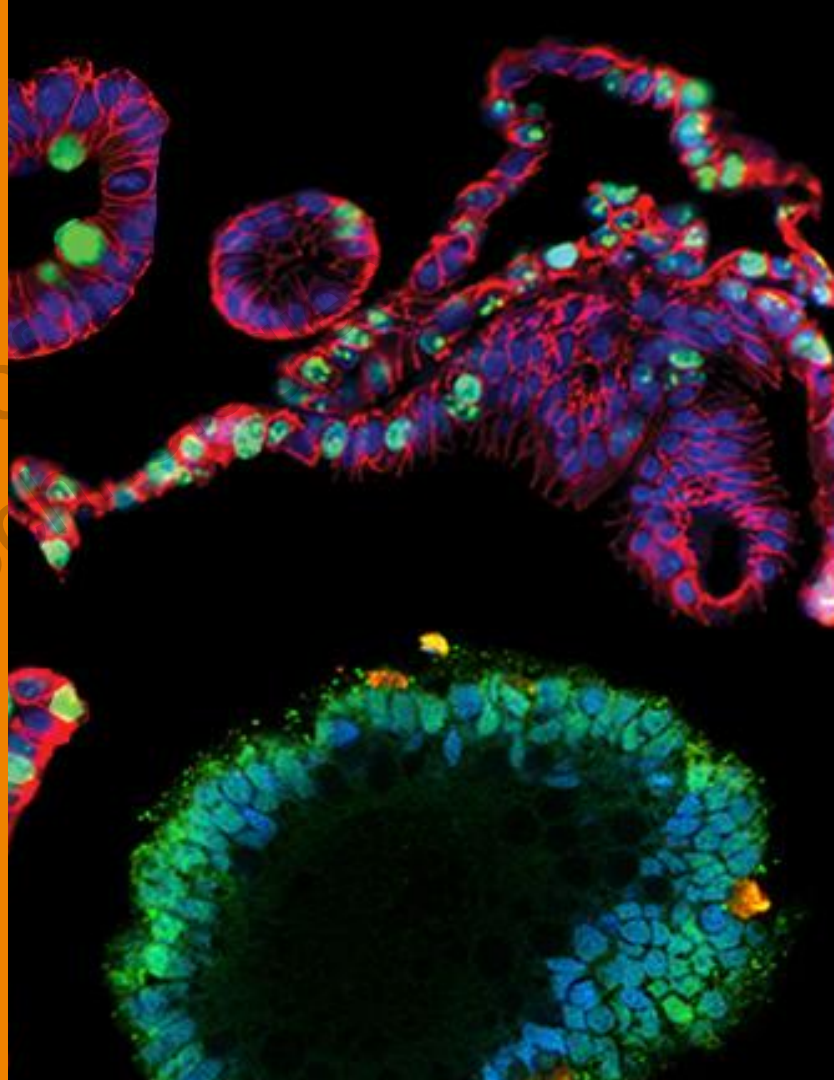
STEMCELL™ TECHNOLOGIES



Learning Objectives

After this session, you should be able to:

- Understand the key techniques for characterization and function of intestinal cultures
- Be aware of the key research applications for human intestinal organoids and possible experimental endpoints

Outline

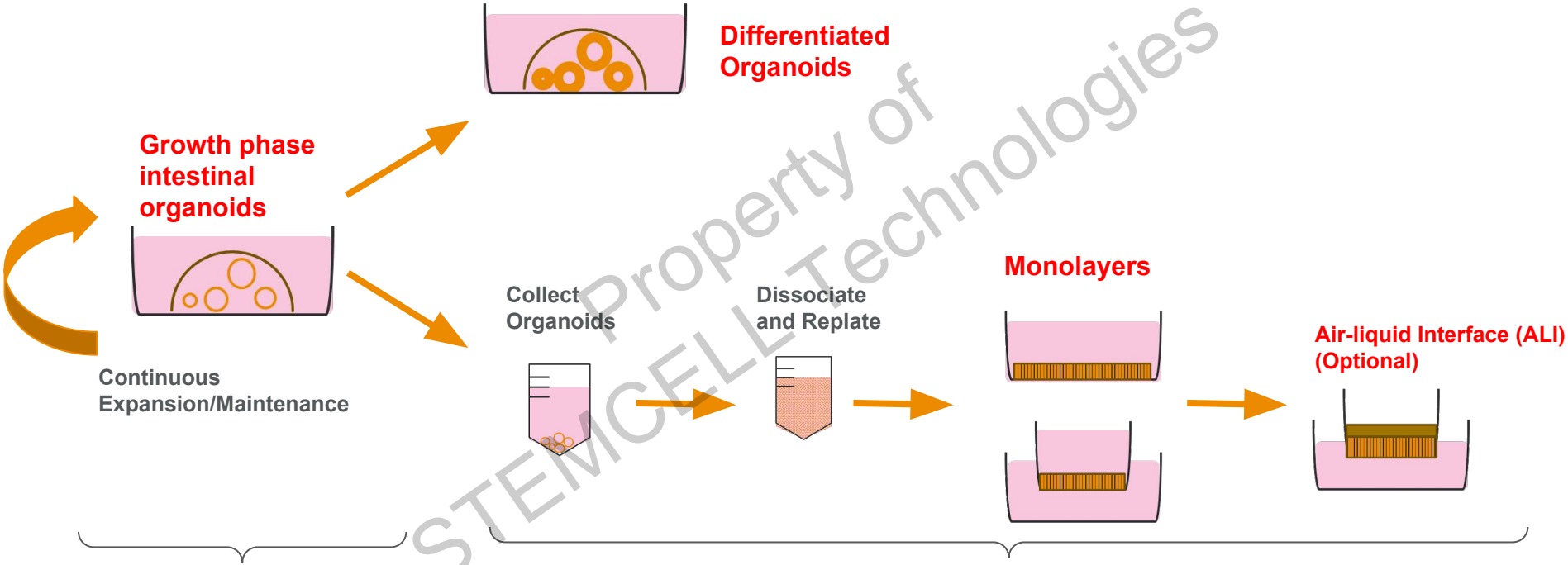
1. Characterization and Function of Intestinal Cultures

2. Application Highlights

    - Nutrient Absorption and Metabolism
    - Disease Modeling (Inflammatory bowel disease, colorectal cancer)
    - Cancer Research
    - Compound Screening and Toxicity Applications
    - Inflammation and Immunity
    - Co-cultures
    - Organ-on-a-Chip

Section 1 Characterization and Function of Intestinal Cultures

Different Culture Formats for Human Intestinal Organoids



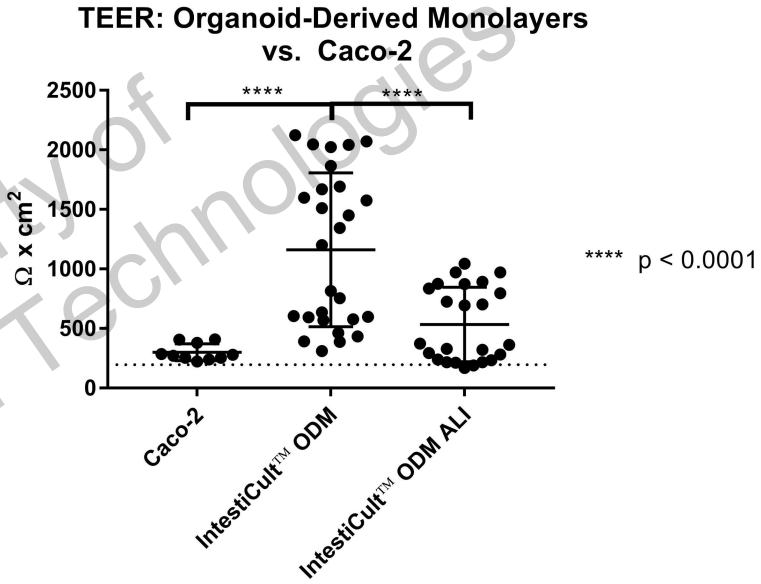
IntestiCult™ Organoid Growth Medium (Human) (OGM)

IntestiCult™ Organoid Differentiation Medium (Human) (ODM)

Transepithelial Electrical Resistance (TEER) in Monolayers/ALI Cultures

TEER is an effective measurement of monolayer integrity, thickness and tight junctions.

- **Qualitatively** measures the general health of cell monolayer during development
- **Quantitatively** measures cell confluence and barrier integrity



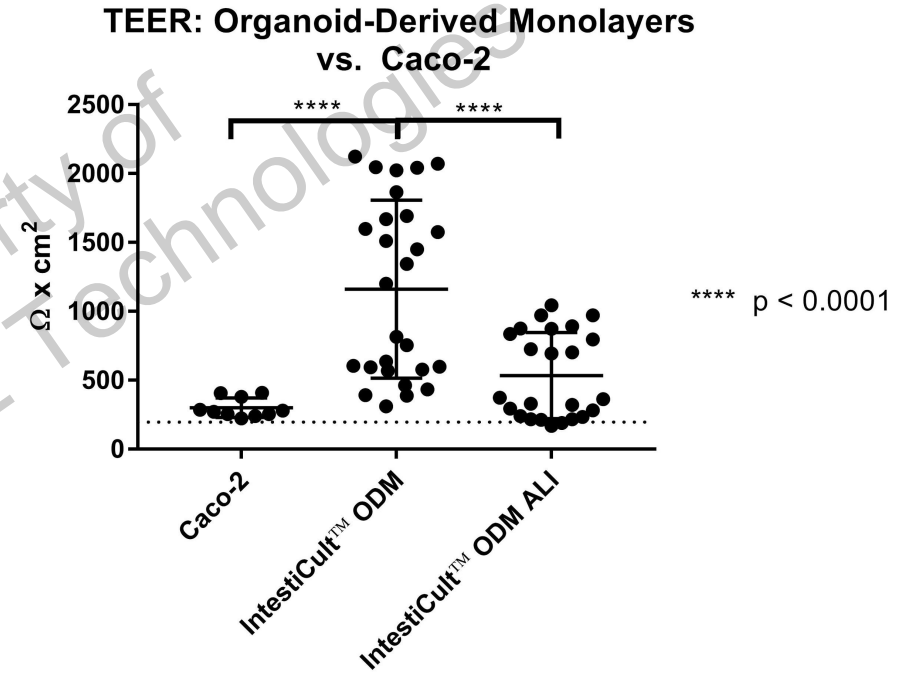
Differentiated organoid-derived monolayers grown as a submerged monolayer (IntestiCult™ ODM Monolayer), or at the ALI (IntestiCult™ ODM ALI), show higher TEER values as compared to Caco-2 cultures.

Technical Video

How to Perform a TEER Measurement to Evaluate Epithelial Barrier Integrity in ALI Cultures

Characterization of TEER in Monolayers/ALI cultures

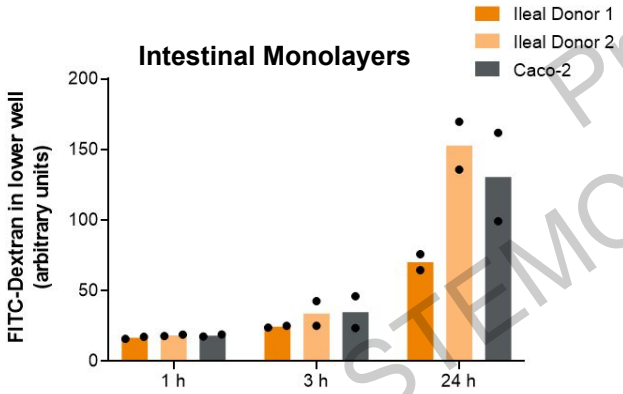
- TEER is an effective measurement of monolayer **integrity**, **thickness,** and **tight junctions**.
- Caco-2 cell lines are the common model used for intestinal studies.
- Monolayer cultures grown with IntestiCult™ ODM (Human) offer substantially **higher** TEER readings than Caco-2 cell cultures.
- Increased secretory cell differentiation in ALI monolayer cultures reduces overall TEER values of the monolayer.



FITC-Dextran Permeability Assay

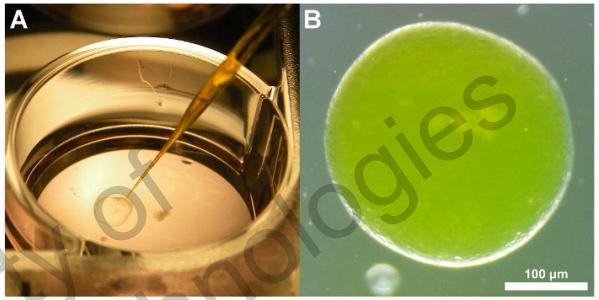
FITC-Dextran Permeability Assay is a measure of intestinal permeability.

- Dextran is a large polysaccharide, ranging in size from 3 to 2000 kDa, which is normally unable to cross an intact epithelial barrier.
- FITC fluorescently-labelled dextran (FITC-dextran) can be used to track changes in epithelial barrier permeability. following exposure to bacteria, screening compounds, etc.

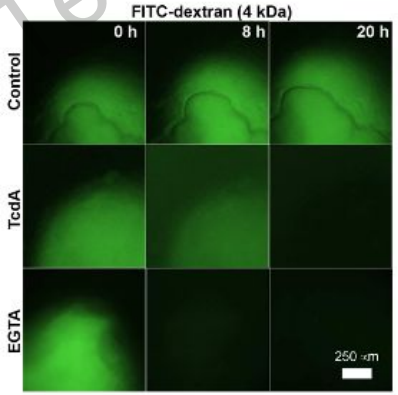


A 4 kDa FITC-dextran added at a concentration of 100ug/mL apically and sampled from the basolateral chamber after 1, 3 and 24 h

Intestinal Organoids



FITC-dextran microinjected into the lumen of human intestinal organoids



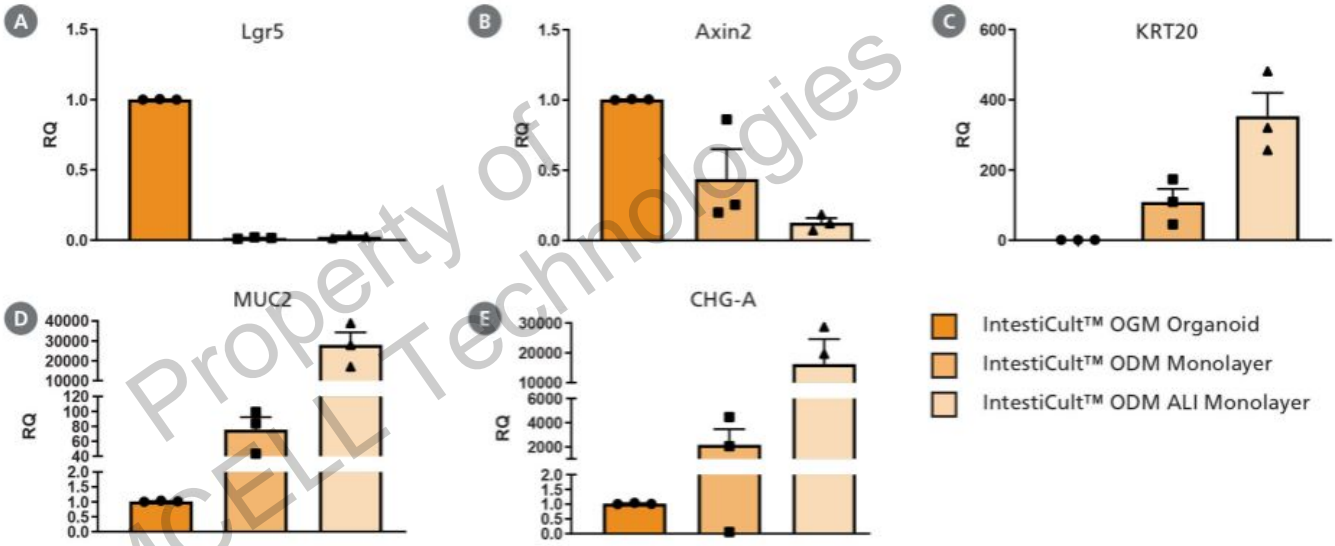
Measure of change in intestinal permeability using FITC-Dextran in response to Clostridium difficile toxin TcdA and EGTA (chelator)

Hill et al. (2017) Real-time Measurement of Epithelial Barrier Permeability in Human Intestinal Organoids. J Vis Exp (130): e56960.

Quantitative PCR (qPCR)

Quantitative PCR (qPCR) is a PCR-based technique that couples amplification of a target DNA sequence with quantification of the concentration of that DNA species in the reaction.

- Involves RNA extraction
- Enables detection and quantification of RNA
- Used for looking at relative changes in gene expression



Changes in gene expression as measured by RT-qPCR.
Relative quantification (RQ) for each marker is shown relative to actB and TBP housekeeping genes and normalized with respect to undifferentiated organoids grown in IntestiCult™ OGM (Human).

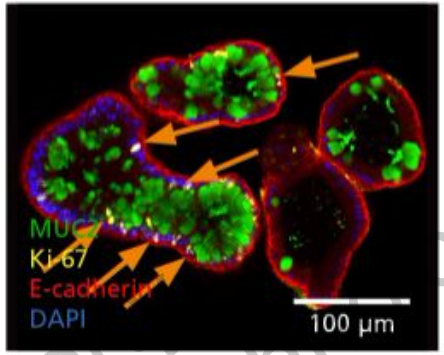
Imaging

Immunocytochemistry (ICC) can detect relevant epithelial markers and assess for markers of differentiation.

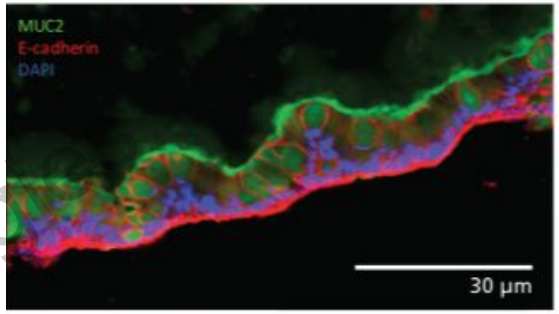
- Enables researchers to evaluate different experimental conditions
  - For instance, it can be used to assess culture marker expression in the presence or absence of a specific drug or pathogen.
- Confirm/characterize the presence of different cell types (proliferative cells vs differentiated cells) in response to change in culture conditions

Whole mount ICC staining

Organoids



Monolayers

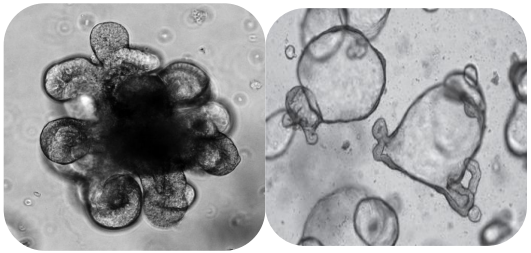


Technical Protocol
Performing Immunocytochemical Staining of Epithelial Organoids

Technical Protocol
How to Perform Immunocytochemistry (ICC) Staining of Epithelial Cells Cultured as Monolayers or at the Air-Liquid Interface

Brightfield Microscopy can be used to observe the shape of organoids

- No preparation or staining required, simple and practical for observation

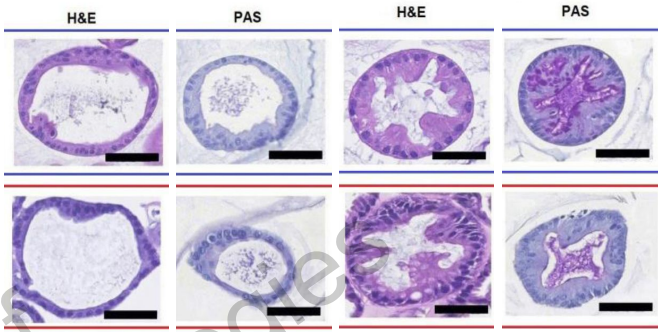


Brightfield microscopy

Imaging

Histology is a common method for looking at “gross tissue morphology”.

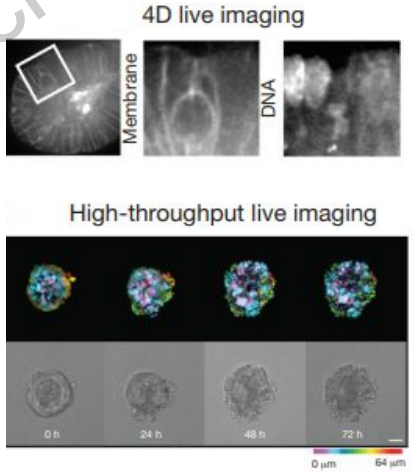
- Hematoxylin and eosin (H&E) staining is a common tissue stain used in histology.
- Periodic acid-Schiff (PAS) is a staining method used to detect polysaccharides.
    - PAS specifically highlights goblet cells.



Bergenheim et al (2020). A fully defined 3D matrix for ex vivo expansion of human colonic organoids from biopsy tissue. Biomaterials (262): 120248.

Live Cell Imaging is used to investigate living cells over a period of time using time-lapse microscopy

- Higher resolution; can be used to look at cellular dynamics
- Real-time; live-cell assays

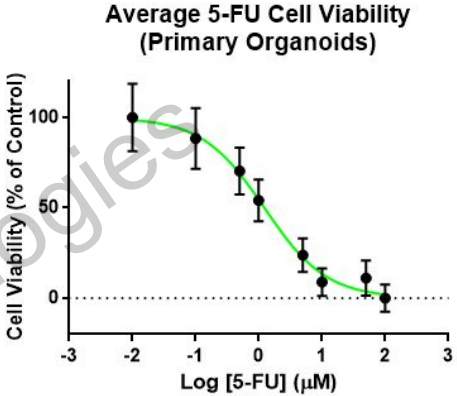


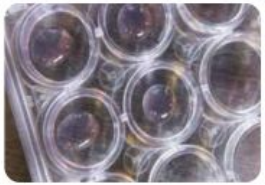
Modified from Rios et al (2018). Imaging organoids: a bright future ahead. Nature methods (15): 24-26.

Cell Viability

Cell Viability assays evaluate the number of viable cells remaining in a culture either **directly** (by live/dead stain) or **indirectly** (by measuring byproducts of either live or dead cells).

- CellTiter-Glo® 3D Cell Viability Assay measures the presence of ATP (a product of live cell metabolism) in culture.
- ATP reacts with luciferin to produce oxyluciferin and light, which can be quantified with a luminometer.





Scale down to 96-well format

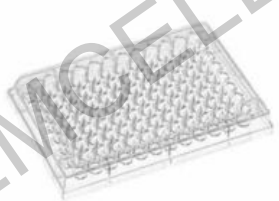
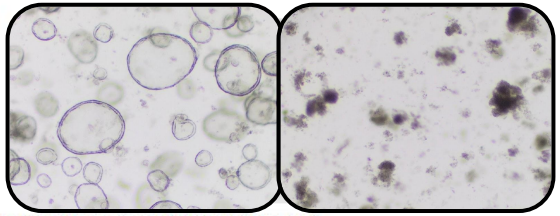


Plate crypts, grow for 2 days

5-day treatment



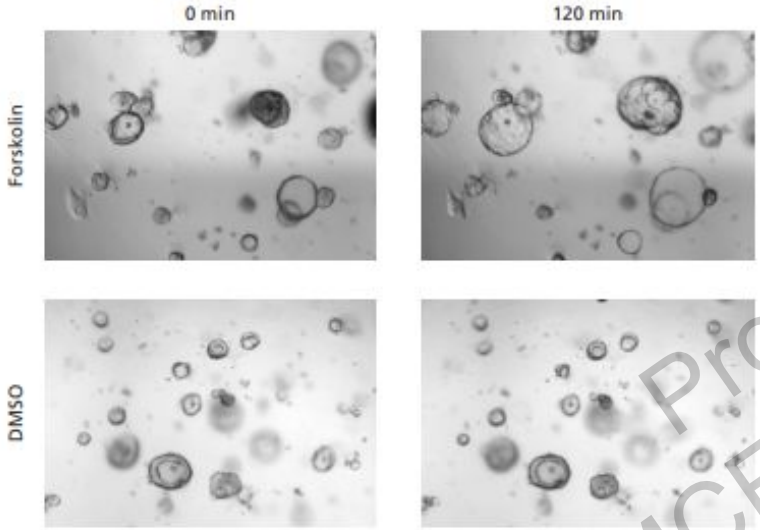
Assess viability via CellTiter-Glo® 3D

Number of days is flexible (donor/culture-dependent)

Schematic of 96 well-human intestinal organoid viability set-up

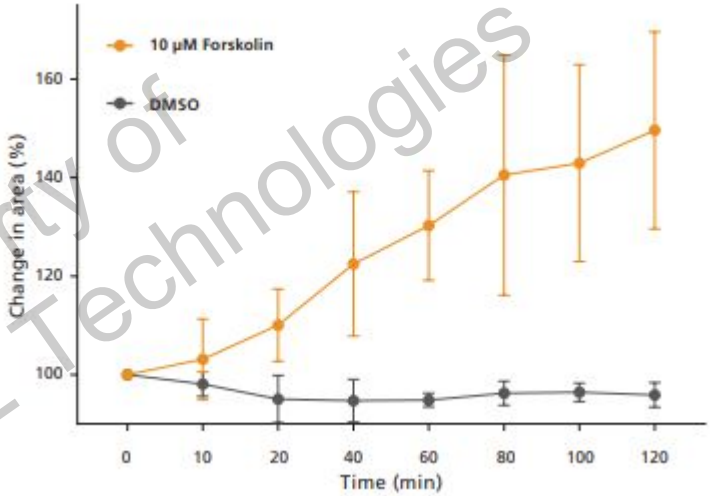
Forskolin-Induced Swelling (FIS) Assay

Intestinal organoids are amenable to functional assays



Forskolin swelling assay with intestinal organoids grown in IntestiCult™ OGM (Human) - Morphology

- Swelling is a function of Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) activity
- Defective CFTR= no swelling



Forskolin swelling assay with intestinal organoids grown in IntestiCult™ OGM (Human) - Quantification

- Can calculate % change in organoid area in response to Forskolin treatment
- DMSO-treated organoids = no change in area (no swelling)

Technical Bulletin: Forskolin-Induced Swelling of Human Intestinal Organoids Grown in IntestiCult™

Summary

- Human intestinal organoids are amenable to a wide range of experimental readouts, which include
  - TEER
  - Molecular Biology (qPCR, Western Blots, ELISA)
  - Imaging (Brightfield, ICC, IF)
  - Cell Viability Assays
  - FITC-Dextran Permeability Assay
  - Forskolin-Induced Swelling (FIS)

Section 2 Application Highlights

Nutrient Absorption and Metabolism

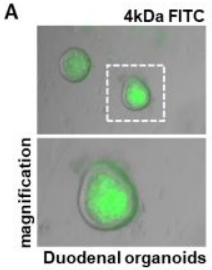
Nutrition and Metabolism Studies

Brief Summary:

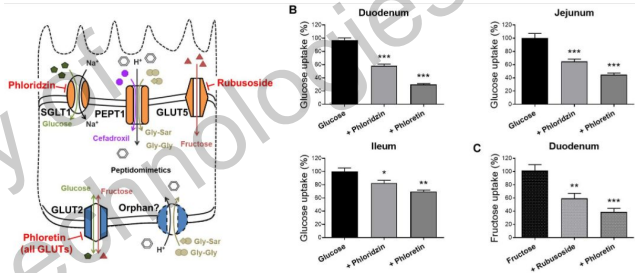
- Used FITC- labeled dextrans to show molecules of a size 4 kDa rapidly reach the luminal compartment of human organoids (similar approach published with murine organoids)
- Highlights the utility of FITC-dextran permeability assay for transport and metabolic measurements

Significant Findings:

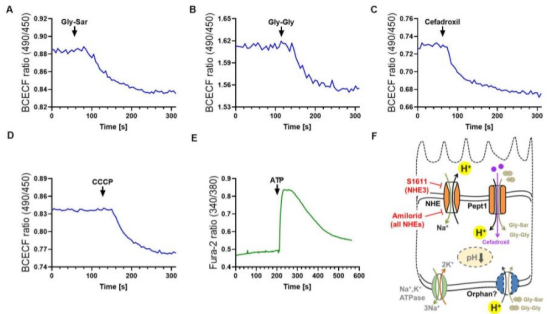
- Organoids allow concurrent study of nutrient and drug transport, sensing and incretin hormone secretion and live-cell imaging of intracellular processes.



Substances	Size (Da)
Sugars	
Glucose	180
Fructose	339
Dipeptides	
Gly-Sar	146
Gly-Gly	132
Drugs	
Cefadroxil	363
P1	840
P2	570
P3	455



Measurement of glucose and fructose uptake in human intestinal organoids



Measurement of peptide transport in human intestinal organoids by looking at intracellular acidification (pH indicator)

Zietek et al. (2020) Organoids to Study Intestinal Nutrient Transport, Drug Uptake and Metabolism - Update to the Human Model and Expansion of Applications. Front Bioeng Biotechnol (8): 577656.

Disease Modeling

Modeling Inflammatory Bowel Disease (IBD)

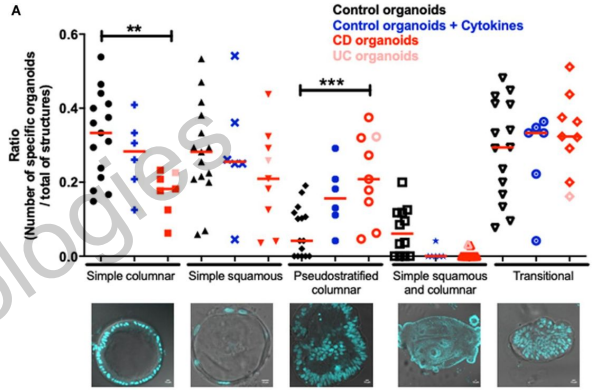
Brief Summary:

- Colonic organoids established from IBD samples
- Morphological and gene expression characterization using primary organoid cultures from IBD patients
- Organoids exposed to different treatments currently used

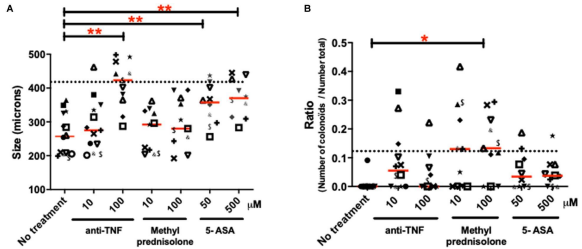
Significant Findings:

- IBD organoid cultures could be used to test the effects of therapeutic options on epithelial biology.
- Organoids could be predictive of the patient's response to treatment, offering an option for personalized medicine.

d'Aldebert et al. (2020) Characterization of Human Colon Organoids From Inflammatory Bowel Disease Patients. Front Cell Dev Biol (8): 363.



Intestinal organoids recapitulate differences in culture morphology between healthy and diseased sample.

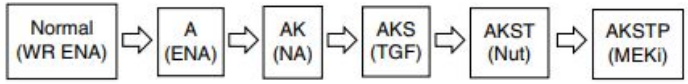


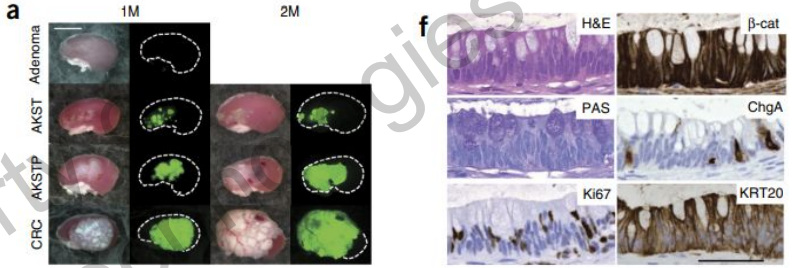
IBD derived organoids show dose-dependent response to all treatments.

Modeling Colorectal Cancer in vitro

Brief Summary:

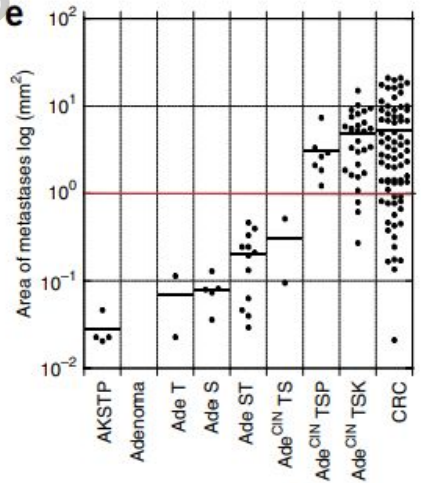
- CRISPR-Cas9 methods to engineer oncogenic mutations in 3D organoids
- Engineered organoids formed tumors after implantation under the kidney subcapsule in mice





Significant Findings:

- Organoids combined with gene editing approaches provide a powerful tool to model “oncogenic transformation” in vitro.



Matano et al. (2015) Modeling colorectal cancer using CRISPR-Cas9-mediated engineering of human intestinal organoids. Nat Med 21: 256-62.

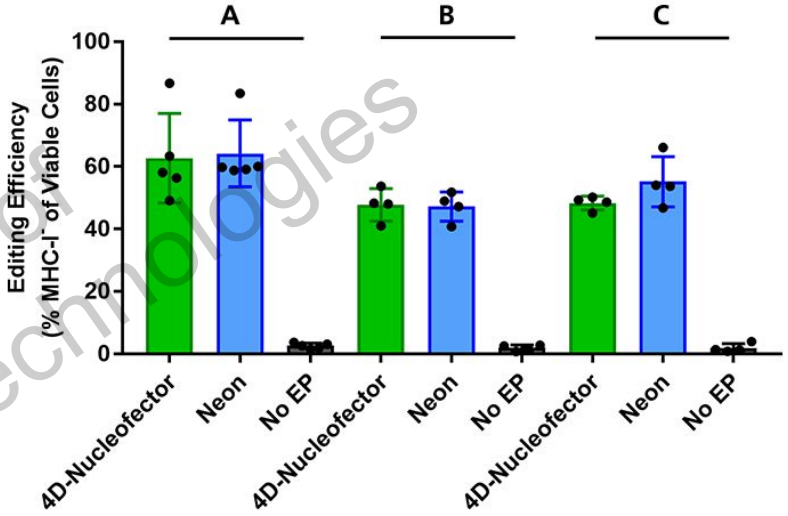
CRISPR-Cas9 Genome Editing of Human Intestinal Organoids

Brief Summary:

- ArciTect™ CRISPR-Cas9 ribonucleoprotein (RNP)-based system for gene editing human intestinal organoids cultured in IntestiCult™ Organoid Growth Medium (OGM)(Human) (Catalog #06010)
- RNP complexes containing ArciTect™ Cas9 Nuclease and ArciTect™ sgRNA targeting the B2M gene were delivered using electroporation
- Editing efficiency monitored by flow cytometry

Significance

- A step by step detailed protocol for applications involving gene editing with human intestinal organoids
- STEMCELL's Guide RNA Design Tool: Single Guide RNA (sgRNA) Design and Order Tool for CRISPR-Cas9 Genome Editing could be used



Editing efficiency as monitored by flow cytometry to detect surface expression of major histocompatibility complex (MHC) class I molecules (MHC-I) using Neon® Transfection System or the Lonza® 4D-Nucleofector™ X Unit.

Technical Bulletin: Genome Editing of Human Pluripotent Stem Cells Using the ArciTect™ CRISPR-Cas9 System

Cancer Research

Cancer Research

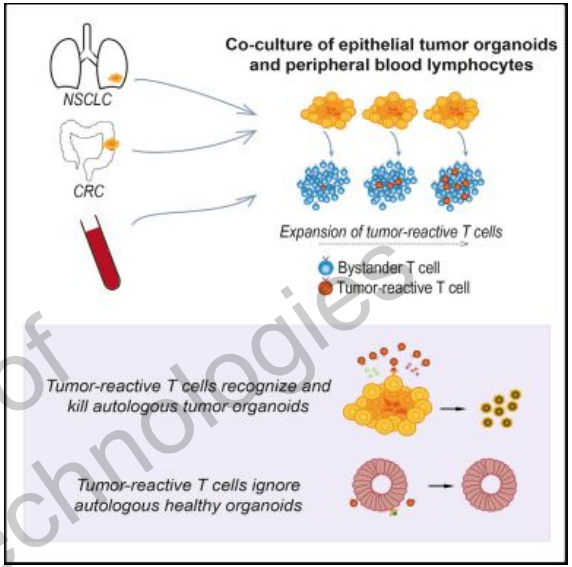
Brief Summary:

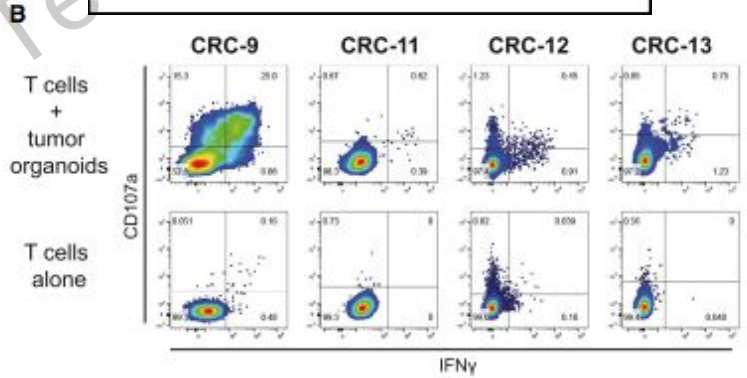
- Stimulation of immune cells with dissociated tumor organoids- T Cells are effectively stimulated to proliferate through exposure to dissociated tumor organoids, but not normal organoids.

- T cell-mediated killing of tumor organoids- the resultant T cells can be used to attack and kill tumor organoids as an in vitro assay of T cell-mediated killing.

Significant Findings:

- Intestinal organoid cultures can be used to expand tumor cells that are very similar to original tumors.
- Intestinal organoids can also act as tumor surrogates for in vitro studies.





Dijkstra et al. (2018) Generation of Tumor-Reactive T Cells by Co-culture of Peripheral Blood Lymphocytes and Tumor Organoids. Cell (174): 1586-1598.e12.

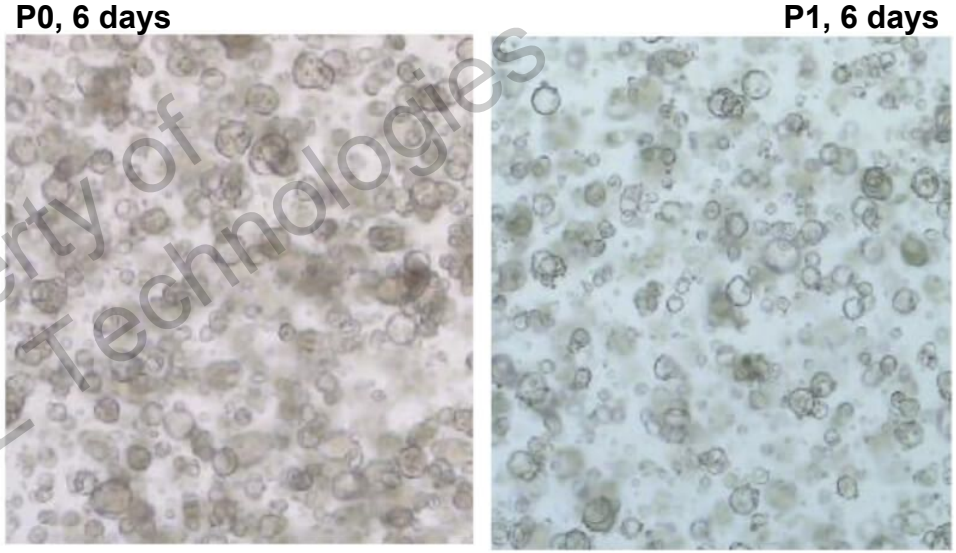
Culturing Cancer-Derived Organoids Using IntestiCult™ OGM (Human)

Wnt-Independent Cancer Organoids
Cancer-derived organoids can be efficiently established and passaged in IntestiCult™ Organoid Growth Medium (OGM; Human) (basal medium alone, mixed 1:1 with DMEM/F-12).

Tumors that do not carry an activating mutation of the Wnt pathway cannot be maintained as organoids using this method.

Key Protocol Steps:

- Isolation of tissue from tumor samples using Gentle Cell Dissociation Reagent (GCDR)
- Plating organoids in IntestiCult™ OGM Human Basal Medium alone
- Growth medium should be replaced every 2 days, and cultures can be passaged every 6 - 12 days



Cancer-derived organoids demonstrated efficient growth both after establishment in IntestiCult™ OGMH, as well as after passaging

Technical Bulletin: Culturing Cancer-Derived Organoids Using IntestiCult™ Organoid Growth Medium (Human)

Compound Screening and Toxicity

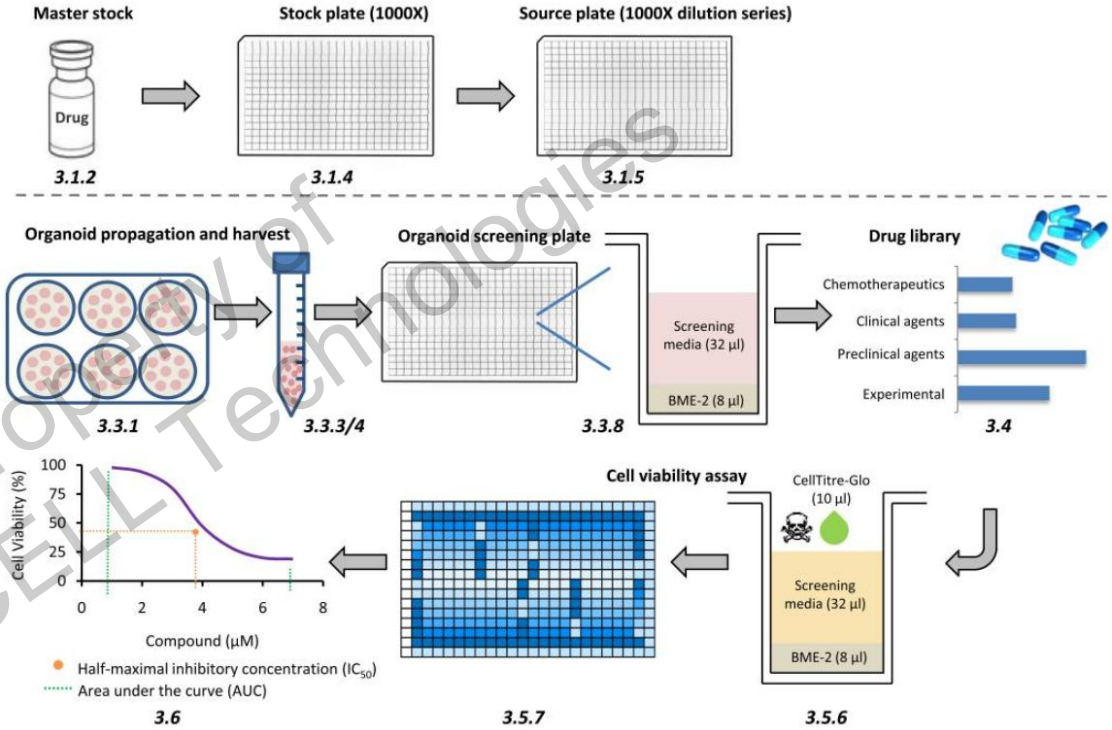
Organoid Compound Screening

Brief Summary:

- Compound screening with human intestinal organoid cultures in 384-well plates using liquid handling robotics (automated)
- **Cell viability readout** used for quantification of organoid response to drugs

Significant Findings:

- Development of a workflow for establishing screen-ready cultures of human cancer organoids



Schematic of organoid-based drug screen workflow

Francies et al. (2021) Drug Sensitivity Assays of Human Cancer Organoid Cultures. Methods Mol Biol (1576): 339-351.

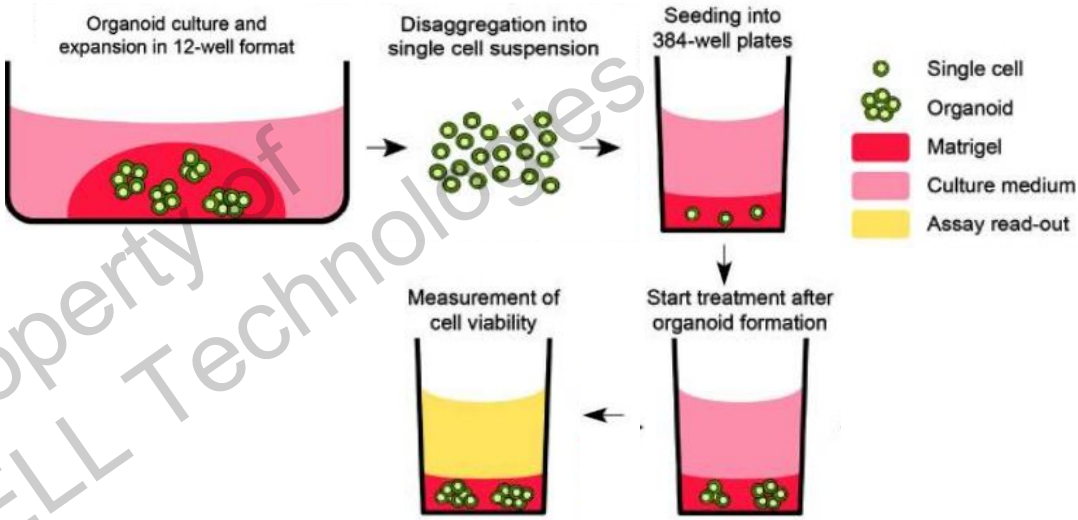
Organoid Compound Screening

Brief Summary

- Culture of human colon cancer organoids cultured in defined medium in 384-well plates
- Plate uniformity evaluation and assessment of assay sensitivity and reproducibility
- Drugs mixed within the media, which diffuses through the matrix to act on organoids

Significant Findings:

- Semi-automated, high-throughput organoid cultures validated for drug sensitivity assays



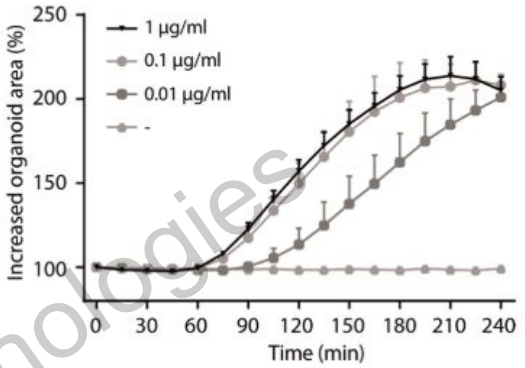
Patient-derived colon cancer organoids established in 384-well plates from single-cell suspension

Boehnke et al. (2016) Assay Establishment and Validation of a High-Throughput Screening Platform for Three-Dimensional Patient-Derived Colon Cancer Organoid Cultures. J Biomol Screen (21): 931-41.

Organoid Compound Screening

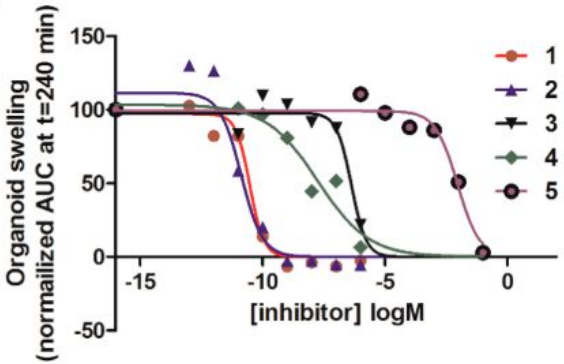
Brief Summary:

- Measurement of **organoid swelling** as assay of toxin-induced diarrheal response
- Dose-response measurements for toxin inhibitory compounds



Significant Findings:

- Organoid swelling provides a simple model system for studying compounds that modulate intestinal diarrheal response.



Zomer-van Ommen et al. (2016) Functional Characterization of Cholera Toxin Inhibitors Using Human Intestinal Organoids. J Med Chem (59): 6968-72.

Inflammation and Immunity

Inflammation and Immunity

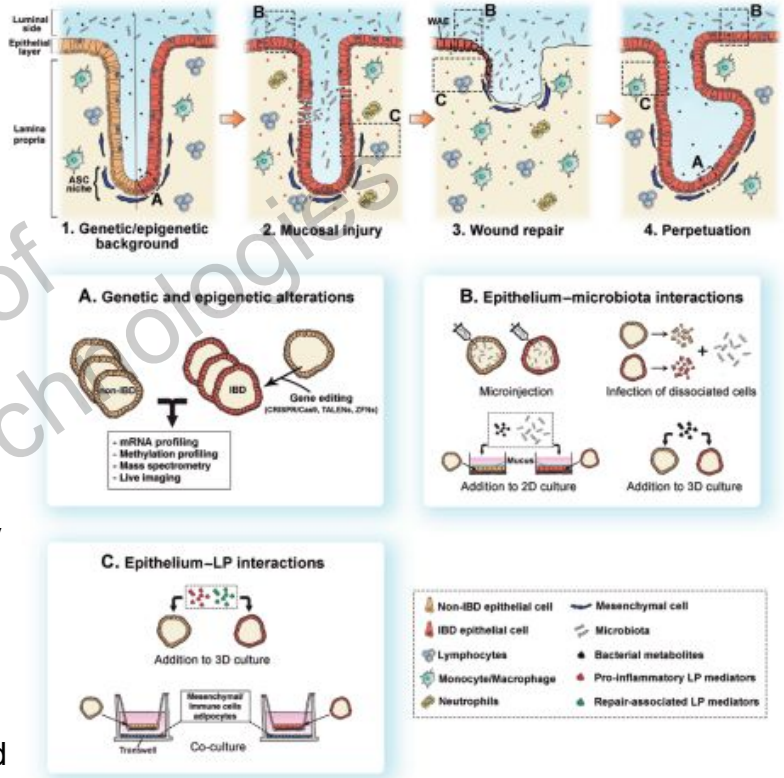
Brief Summary

- Monolayer culture of organoid-expanded epithelial cells
- Co-cultures of organoids or organoid-derived monolayers with microorganisms, stromal cells, or immune cells
- Gene editing in organoids

Significant Findings:

- Organoids are a flexible tool for studying Inflammatory Bowel Disease (IBD), but samples from most actively inflamed areas may have too few active crypts to culture organoids.
- Representation of epigenetic state of sampled tissue in organoids is a major benefit of these cultures for studying inflammatory disease.
- There is a “pressing need” for standardization of intestinal organoid culture techniques to move into clinical applications.

Dotti, I., & Salas, A. (2018). Potential Use of Human Stem Cell-Derived Intestinal Organoids to Study Inflammatory Bowel Diseases. Inflamm Bowel Dis (24): 2501–2509.



There are many emerging techniques for modeling IBD with organoids that drastically increase the experimental toolkit available for IBD research.

Inflammation and Immunity

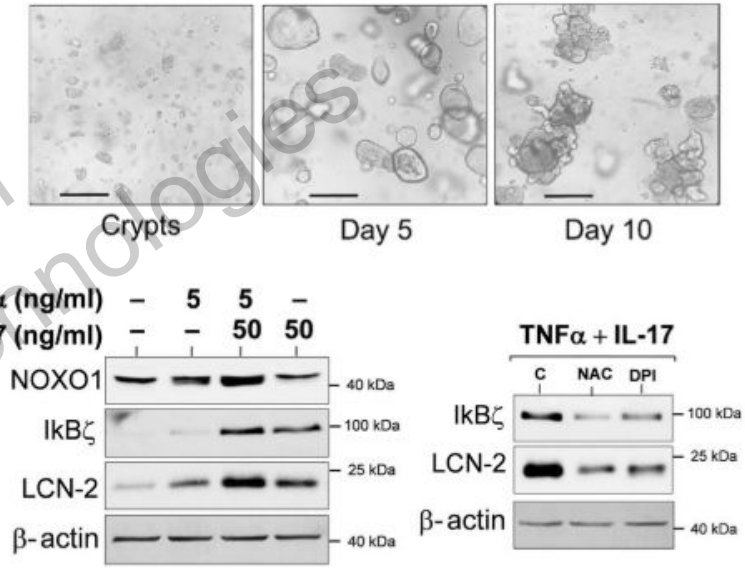
Brief Summary:

- Healthy colon organoids grown in IntestiCult™ OGM (Human)
- Inflammatory response of human intestinal organoids tested on exposure to cytokines in vitro

Significant Findings:

- Organoids provide a model to study intestinal inflammation in normal, human intestinal epithelial cells (as opposed to cell lines or animal models).

Makhezer et al. (2019) NOX1-derived ROS drive the expression of Lipocalin-2 in colonic epithelial cells in inflammatory conditions. Mucosal Immunol (12):117-131.



Intestinal organoids show upregulation of proteins involved in inflammatory response upon exposure to combinations of inflammation signals.

Inflammation and Immunity

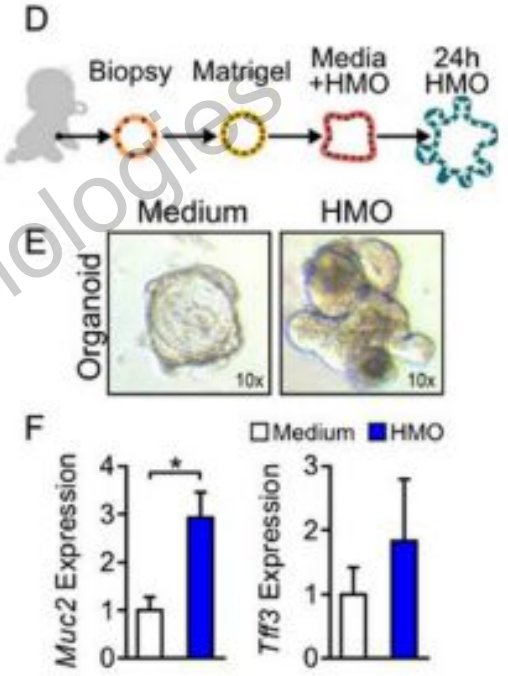
Brief Summary

- Intestinal (ileum) organoids of pre-term neonatal infants established and maintained with IntestiCult™ OGM (Human), ileum not inflamed at time of resection
- Measuring goblet cell function via Muc2 expression in organoids

Significant Findings:

- Organoids provide non-cancerous, human, in vitro model system to study effect of treatment on intestinal epithelium.
- Results in organoids were consistent with the corresponding in vivo data from mouse models.

Wu et al. (2019) Human Milk Oligosaccharides Increase Mucin Expression in Experimental Necrotizing Enterocolitis. Mol Nutr Food Res 63: e1800658.



Intestinal organoids showed expected Muc2 expression increase upon addition of human milk oligosaccharides (HMO).

Co-Culture Models

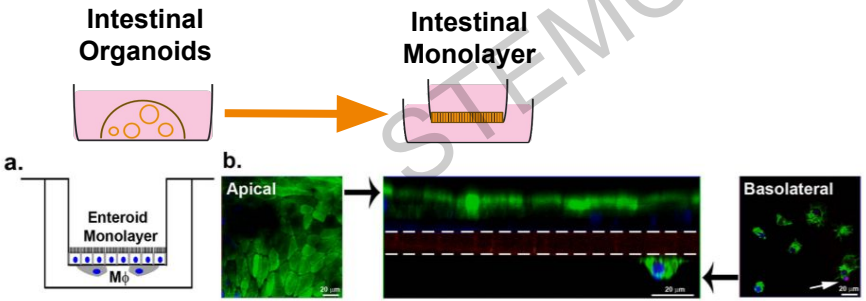
Macrophage-Enteroid Co-Culture Model

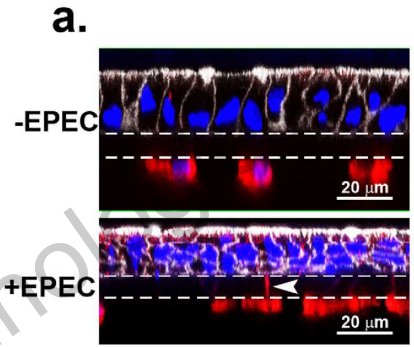
Brief Summary:

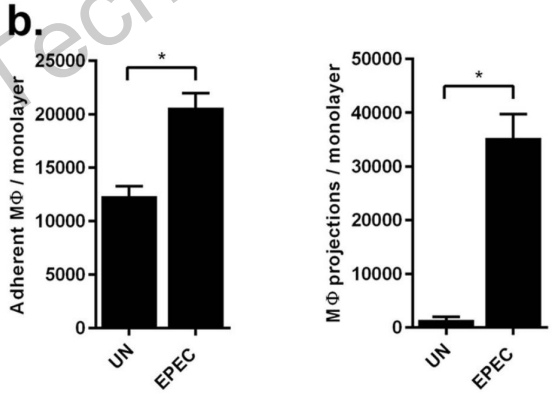
- Intestinal organoids cultured in 2D monolayer format
- Transwell inserts used to establish intestinal and macrophage co-cultures
- Co-cultures infected with enteric pathogens (ETEC and EPEC)

Significant Findings:

- Co-cultures involving intestinal cells and other cell types (immune cells) increases cellular complexity.
- Ex vivo model for studying host-microbial interactions.







Noel et al. (2017) A primary human macrophage-enteroid co-culture model to investigate mucosal gut physiology and host-pathogen interactions. Sci Rep(7): 45270.

Neutrophil-Enteroid Co-Culture Model

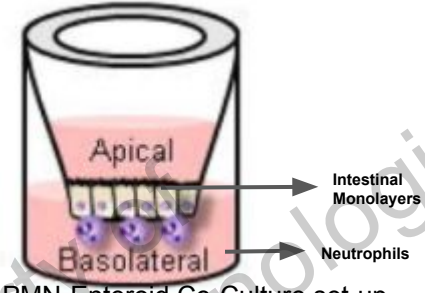
Brief Summary:

- Human ileal organoids seeded onto transwell inserts (apical)
- Polymorphonuclear neutrophils (PMN) isolated from peripheral blood seeded in the basolateral chamber
- Cultures infected with *Shigella*
- Unlike macrophage co-culture, PMN migrated from basolateral to apical side in response

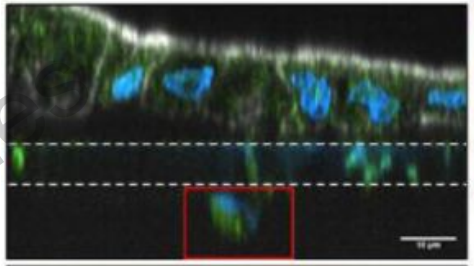
Significant Findings:

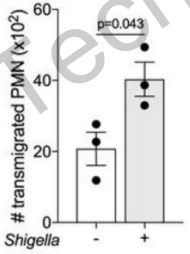
- Organoid-derived monolayers in a co-culture setup offer a **translationally relevant ex vivo model** to study epithelial physiology, host cell interactions, and innate responses to enteric organisms.

Lemme-Dumit et al. (2020) Host-cell interactions and innate immune response to an enteric pathogen in a human intestinal enteroid-neutrophil co-culture. bioRxiv:281535.

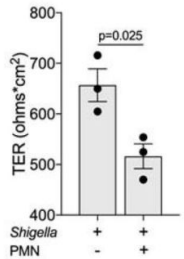


PMN-Enteroid Co-Culture set-up

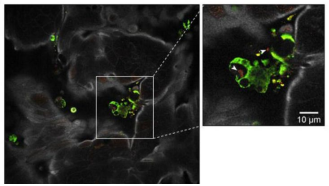




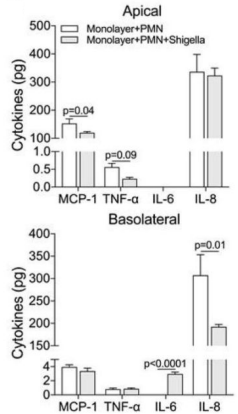
PMN migration post-infection



Increased permeability post-infection (TEER)



Neutrophils (green) apical with Shigella (red)



Cytokine production in apical and basal chambers post-infection

Organ-on-a-Chip

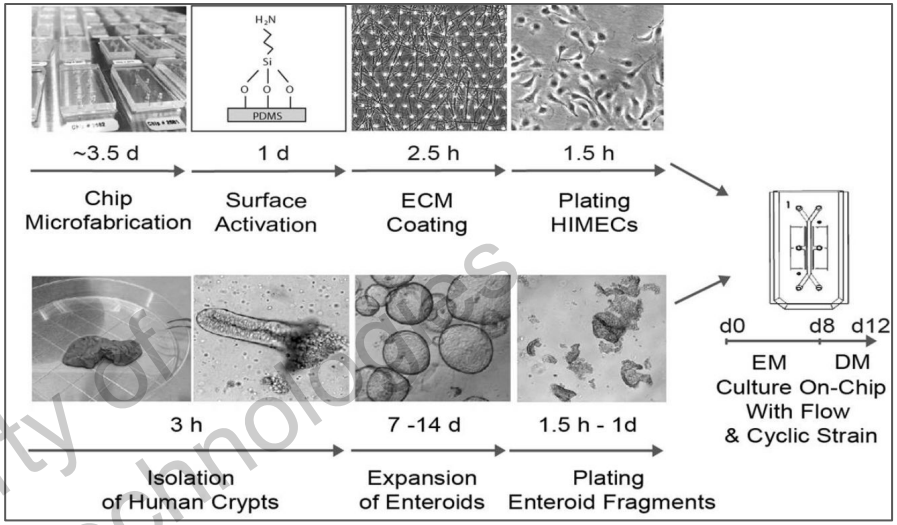
Organ-on-a-Chip

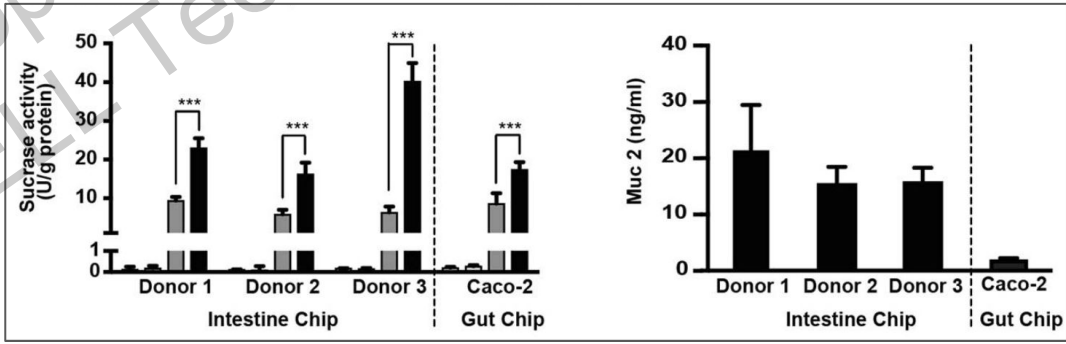
Brief Summary:

- Culture of intestinal cells in gut-on-chip system
- Intestinal organoids used to seed chips
- Chip profiled is from Emulate Inc.

Significant Findings:

- Organ-on-a-chip, established with cells expanded as intestinal organoids, generated a highly physiological system with the ability to effectively model development, function, homeostasis, and disease.





Functional assays of organ-on-a-chip systems derived from intestinal organoids vs. Caco-2 cell line.

Kasendra et al. (2018) Development of a primary human Small Intestine-on-a-Chip using biopsy-derived organoids. Sci Rep (8): 2871.

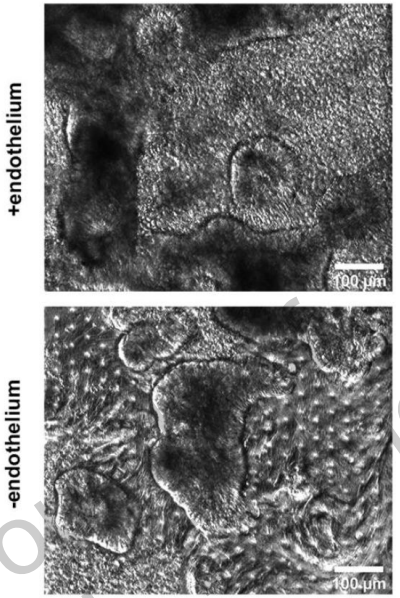
Organ-on-a-Chip

Brief Summary:

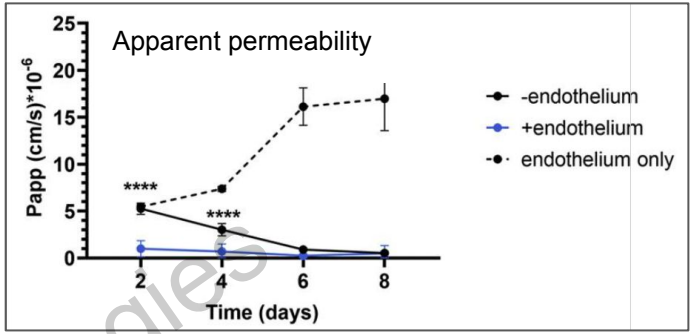
- Colon Intestine-Chip (Emulate) seeded with human colon crypt-derived epithelial and prin microvascular endothelial cells (co-cultures)
- Colon Intestine-Chip treated with cytokines li IFNγ and IL-22 (barrier disruption model)
- Model for intestinal epithelial barrier (leaky gu

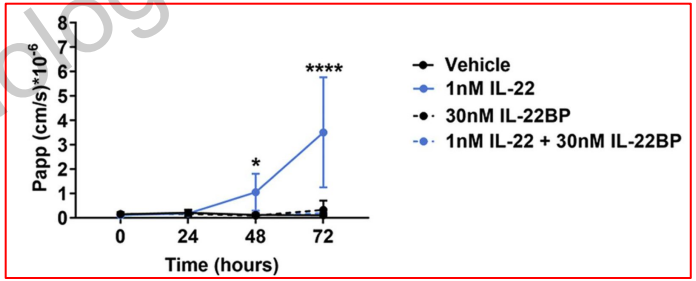
Significant Findings:

- Colon Intestine-Chip recapitulates complex intestinal microenvironment and offers a physiologically relevant model sensitive to established barrier-disruption assays.

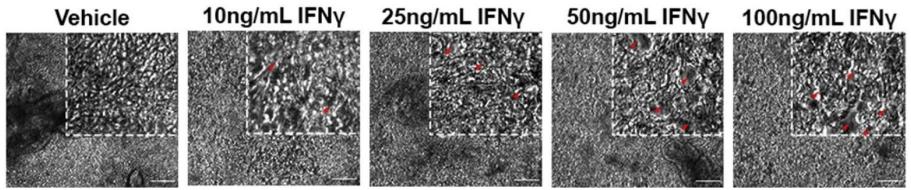


Endothelial cells support tight epithelial barrier.





IL-22 triggers loss of epithelial barrier integrity; rescued by the presence of its soluble receptor IL22BP (redbox)



IFNγ shows barrier disruption in a dose-dependent manner.

Apostolou et al. (2021) A Novel Microphysiological Colon Platform to Decipher Mechanisms Driving Human Intestinal Permeability. Cell Mol Gastroenterol Hepatol (12): 1719–1741.

Summary

Human Intestinal Organoids are amenable to a wide range of research applications and experimental readouts.

Human Intestinal Organoids are important tools for:

- personalized medicine
- prediction of drug responses in patients (cancer, cystic fibrosis, IBD)

Human Intestinal Organoids offer a great model for basic studies such as:

- drug development and toxicology testing
- complementing and reducing animal testing
- metabolic research
- a model of the intestinal epithelium
- host-microbe interactions
- epithelial-immune cell interactions