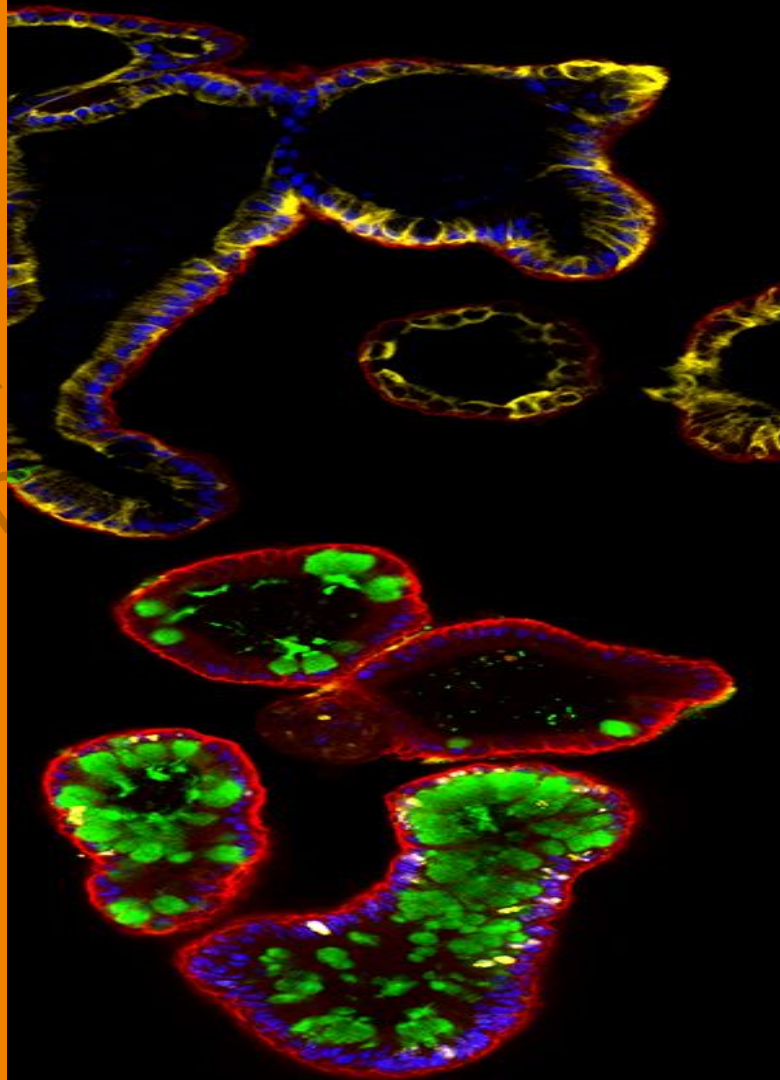
Introduction to Intestinal Biology

Lecture 1

Presenter
Kiran Bhullar
Scientist, Scientific Support, Epithelial Cell Biology

STEMCELL™ TECHNOLOGIES



Learning Objectives

After this session, you should be able to:

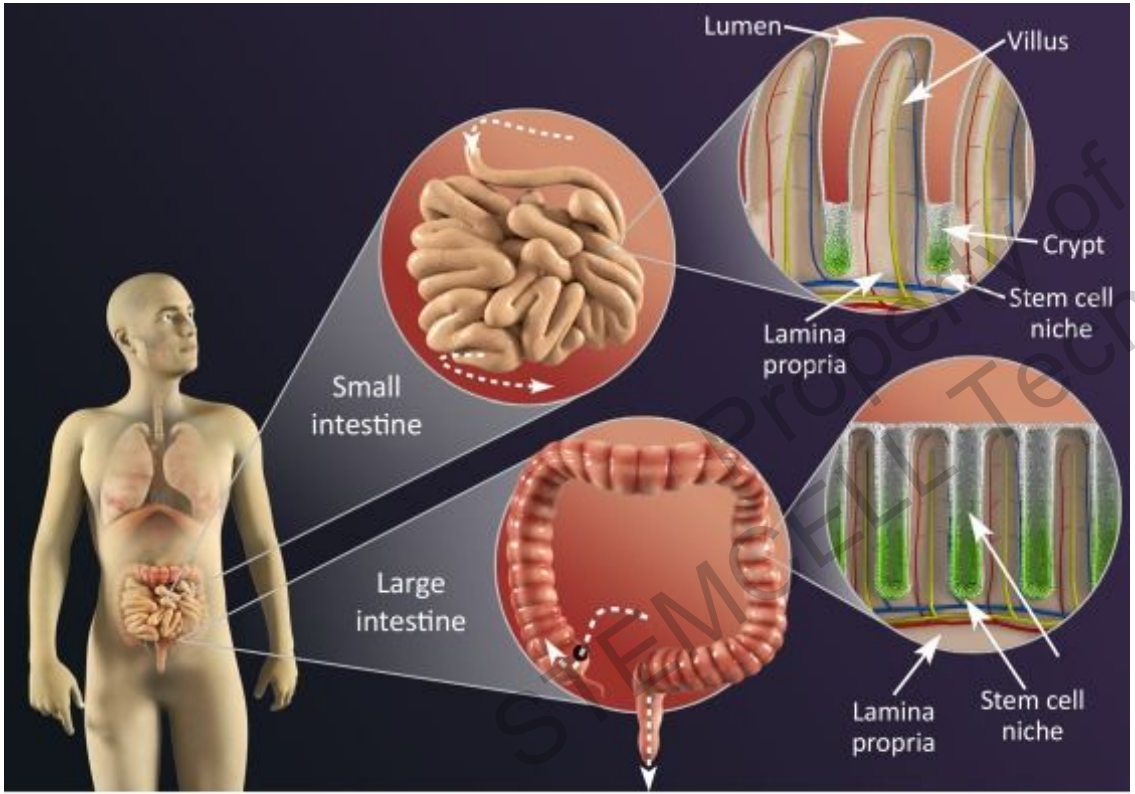
- Describe the intestinal research landscape and the history of intestinal organoid systems
- Describe different intestinal culture methods

Outline

1. Introduction to Intestinal Biology
2. Introduction to Intestinal Organoids
3. Intestinal Model and Culture Systems

Section 1 Introduction to Intestinal Biology

Overview of Gastrointestinal Tract



Key Features:

- The functional unit of the intestine → crypt-villus axis
- Clonal conveyor belt → shed every 7-10 days in humans
- Architecture shapes signaling → defines function

Dutton et al. (2019),Trends Biotechnol.

Architecture of Small Intestine vs Colon

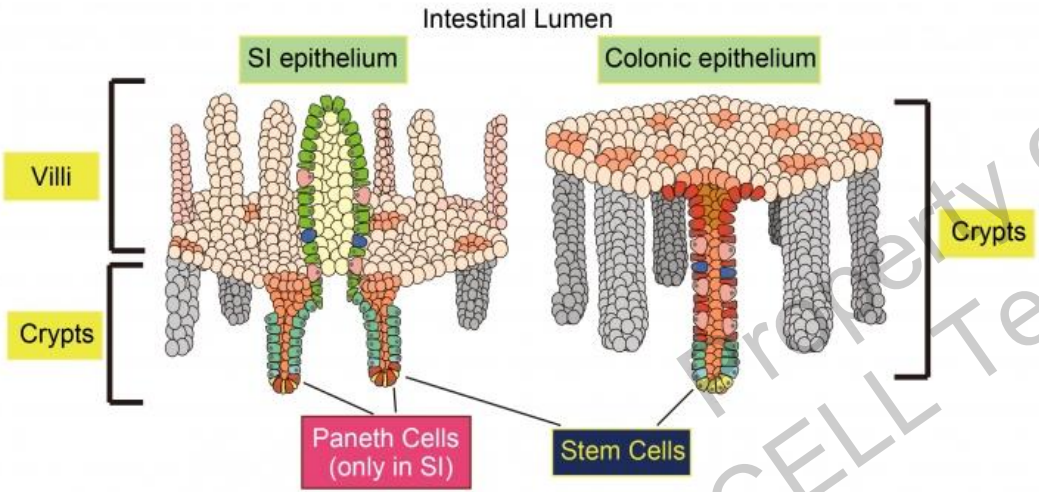


Figure 1 Small Intestinal (SI) and Colonic Epithelia

Press release for Yui et al. (2012), Nature Medicine

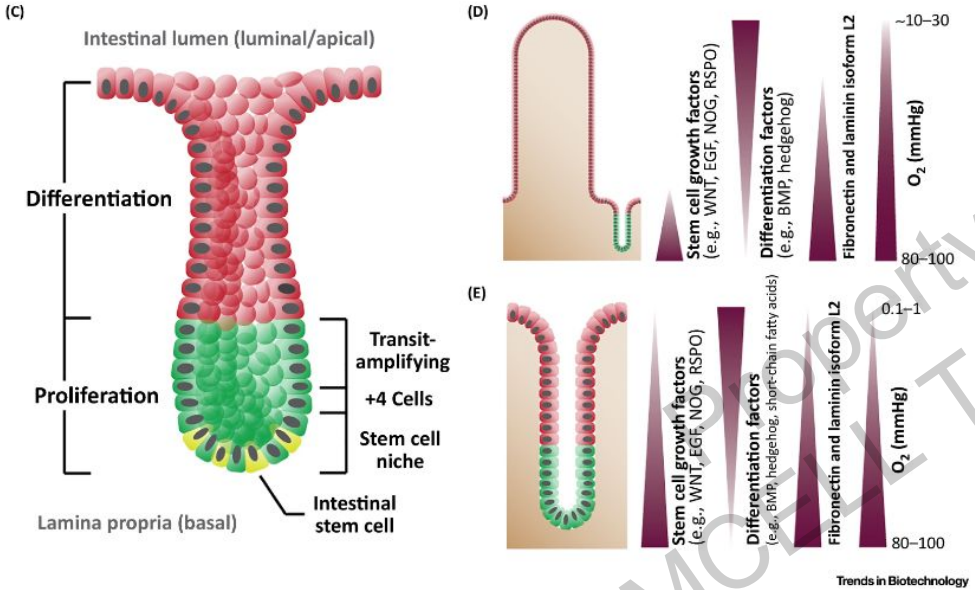
General principle of intestinal epithelium is the same in small intestine and colon:

- Structure of the GI tract varies greatly from region to region
- Common features in the overall "organization" of the intestinal tissue

Features of the colonic epithelium:

- Contains only crypts, no villi
- Stem cell function maintained by Reg4+ cells (not Paneth Cells) in vivo
- Functions primarily to reabsorb fluids
- Site of most intestinal cancers and therefore highly studied

Intestinal Signaling Gradients and Cell Types



Crypt base:

- **High Wnt and EGF signaling, low BMP signaling**
- Favors Paneth cells (SI), Reg4+ cells (colon), Transient Amplifying (TA) cells, and LGR5+ stem cells
- Promotes rapid proliferation

Villus:

- **Wnt and EGF signaling low, high BMP**
- Favours Enterocytes (70%), Goblet cells (7-20%), Enteroendocrine cells (1%), and other cell types (1%)
- Increased functional diversity

Dutton et al. (2019), Trends Biotechnol.

Wnt: Amalgam of Wingless:integration site
EGF: Epidermal growth factor
BMP: Bone morphogenetic protein

Intestinal Epithelial Crypt

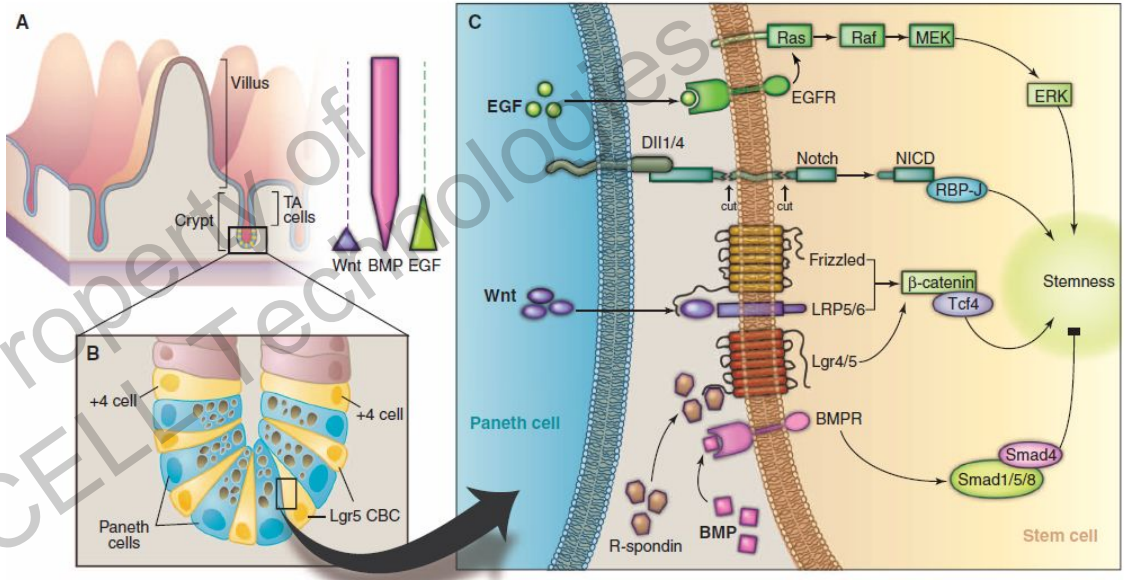
Stem Cell Niche

The intestinal crypt base constitutes a stem cell niche that provides the signals required for the LGR5^{+} stem cells to retain their capacity to self-renew and regenerate the intestinal epithelium.

The niche incorporates spatial gradients of Wnt and EGF that modulate BMP signaling to maintain stemness properties.

Key Signaling Pathways of the Intestinal Stem Cell Niche

- EGF
- Wnt
- BMP



Sato and Clevers. (2013), Science

The Intestinal Epithelium

Cellular Make-Up and Organization

The adult intestinal epithelium is primarily composed of six cell types arranged in a crypt-villus structure.

Convenient Model System

- Rapidly renewing tissue
- Existing detailed knowledge on cell lineages and function
- Convenient model to study adult stem cells, and epithelial cell biology (in addition to intestine-specific applications)

Enterocytes
absorb nutrients from intestinal contents; majority of intestinal epithelium

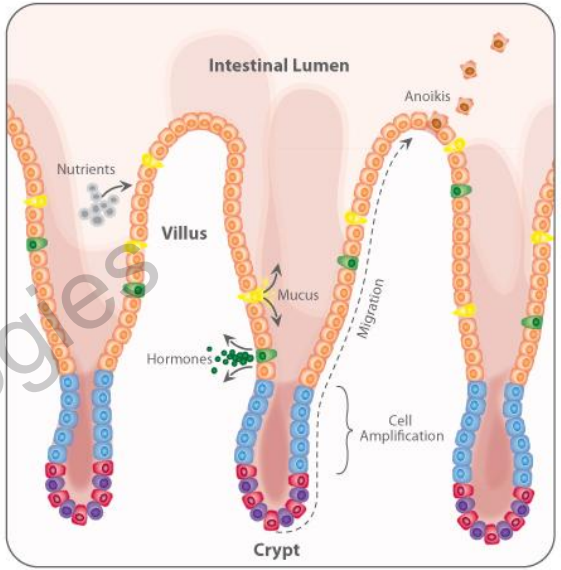
Enteroendocrine cells
secrete hormones into the body to help regulate nutrient metabolism

Goblet Cells
secrete mucus into intestinal lumen; defense against infections

Transit Amplifying Cells
partially differentiated cells that will give rise to the terminally differentiated cells of the villus domain

Paneth Cells
intercalated with the intestinal stem cells; produce intestinal stem cell niche factors; antimicrobial production

Intestinal Stem Cells
give rise to all other intestinal epithelial cell types. This population is LGR5^{+}

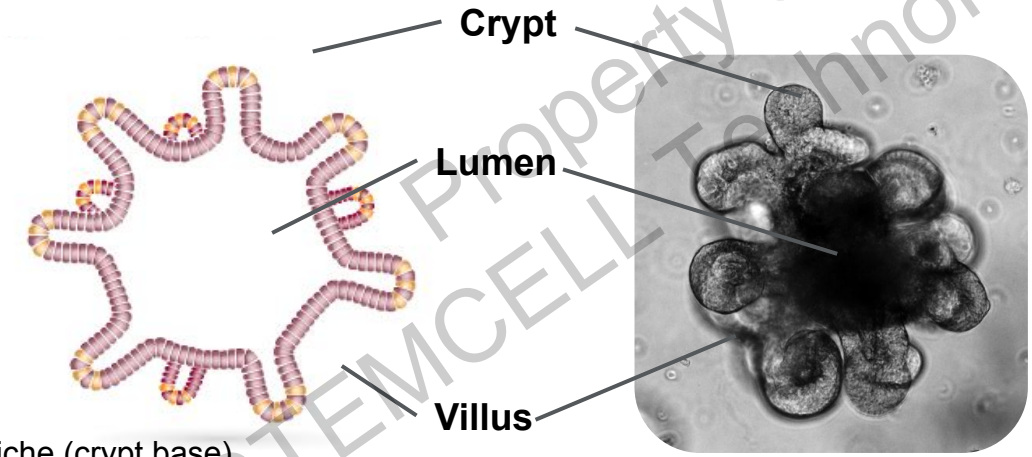


Enterocytes
Enteroendocrine cells
Goblet cells
Transit Amplifying Cells
Paneth Cells
Intestinal Stem Cells

Section 2 Introduction to Intestinal Organoids

Intestinal Organoids - In Vitro Model System for Intestinal Research

Intestinal organoids are three-dimensional (3D) in vitro tissue models that incorporate many of the physiologically relevant features of in vivo intestinal tissue. These features include a polarized epithelial layer surrounding a functional lumen.



The stem cell niche (crypt base)

- **High Wnt signaling, low BMP signaling**

Differentiated epithelium (villus)

- **Low Wnt signaling, high BMP signaling**

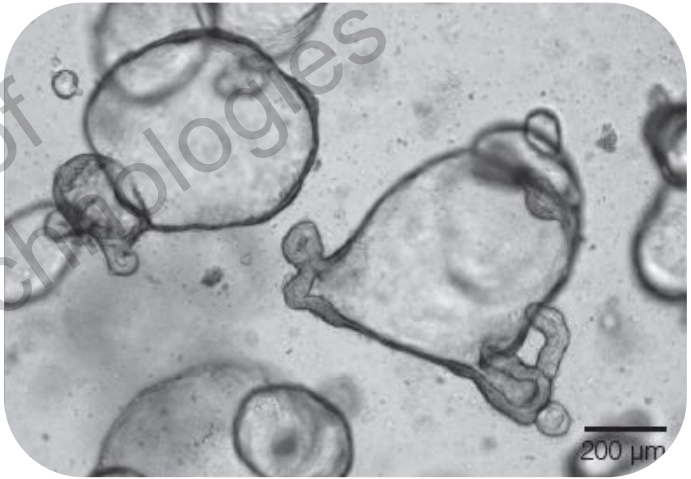
Intestinal Organoid Functionality

Flexible Research Tools

Intestinal organoids have many features that make them attractive model systems.

Organoid Features

- **Recapitulates cellular complement of the in vivo intestine**
- **Genetically identical to source tissue**
- **Ability to expand and maintain in long-term culture**
- **Suitable for cryopreservation and biobanking (allows researchers to leverage genetic diversity of the population, leading to more realistic preclinical data)**



Intestinal Organoid History: Mouse

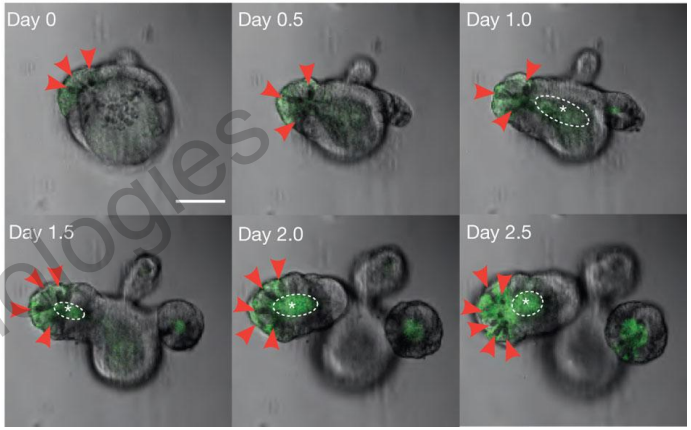
Clevers Lab Pioneering Publication

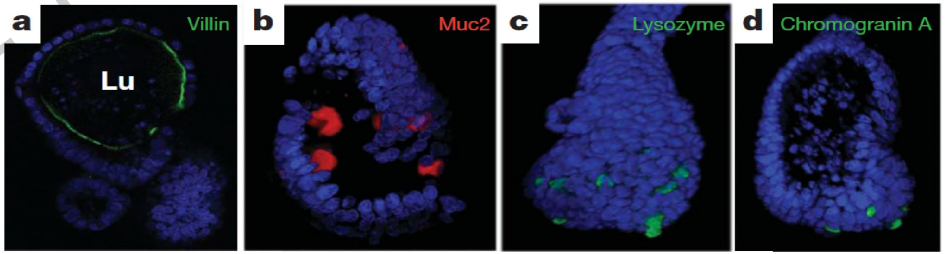
In 2009, Toshiro Sato and Hans Clevers published the first methods for culturing mouse intestinal epithelial organoids from **adult mouse intestinal stem cells.**

This sprang from the group's initial research on the factors that make up the intestinal stem cell niche.

General process

- **Isolate crypts (containing stem cells and paneth cells)**
- **Embed in extracellular matrix (Matrigel®)**
- **Expose to medium containing stem cell niche factors**





Villin: Enterocytes | Mucin 2: Goblet cells | Lysozyme: Paneth cells | ChgA: Enteroendocrine cells

Sato et al. (2009), Nature

hub Hubrecht Organoid Technology

STEMCELL™ TECHNOLOGIES

Intestinal Organoid History: Human

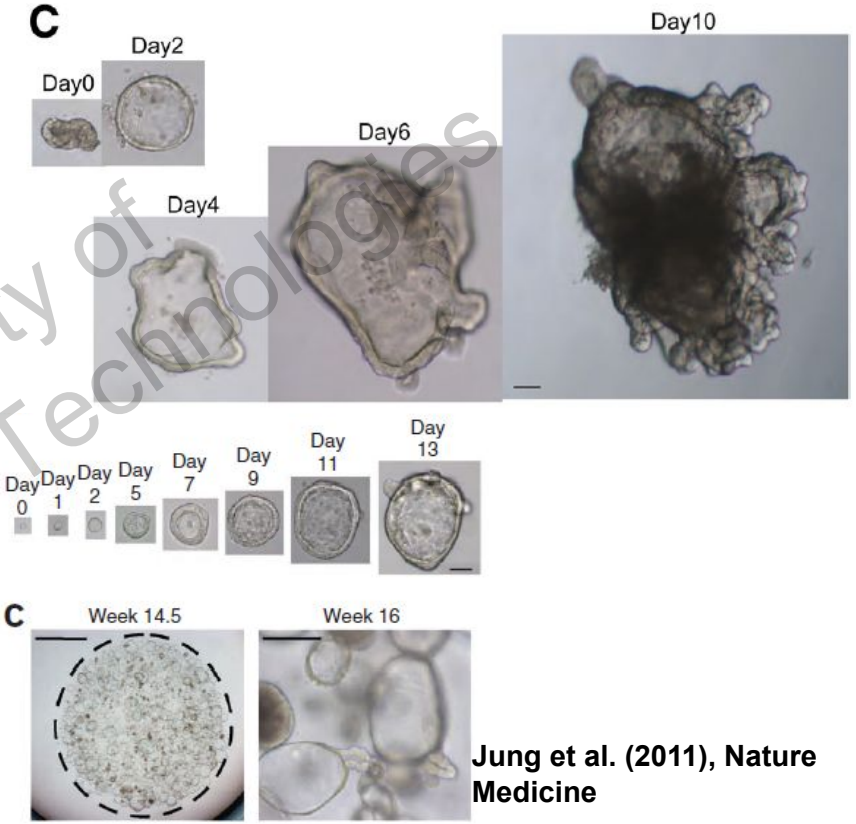
Follow-Up Paper for Human Intestinal Organoids

Sato et al. (2011), Gastroenterology paper described the human adult stem cell (ASC)-derived intestinal organoid system using a similar methodology, with different medium requirements from the mouse system.

General process

- **Isolate crypts (containing stem cells and paneth cells)**
- **Embed in extracellular matrix (Matrigel®)**
- **Expose to medium containing stem cell niche factors**

Overall, very similar methodology and principles between mouse and human workflows



Jung et al. (2011), Nature Medicine

hub Hubrecht Organoid Technology

STEMCELL™ TECHNOLOGIES

Section 3 Intestinal Model and Culture Systems

Intestinal Model Systems

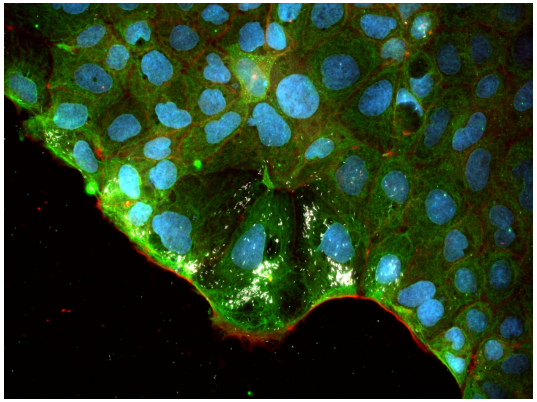
Several model systems are commonly used to model the intestine for a variety of applications:

Intestinal Research Methodologies

- **Intestinal cell lines (eg. Caco-2, HT29)**
- **In vivo studies in mouse models**
- **In vitro culture of primary intestinal cells (primary organoid and organoid-derived monolayer systems)**
- **Examination of surgical or cadaveric samples (ex-vivo)**

Intestinal Cell Culture Systems

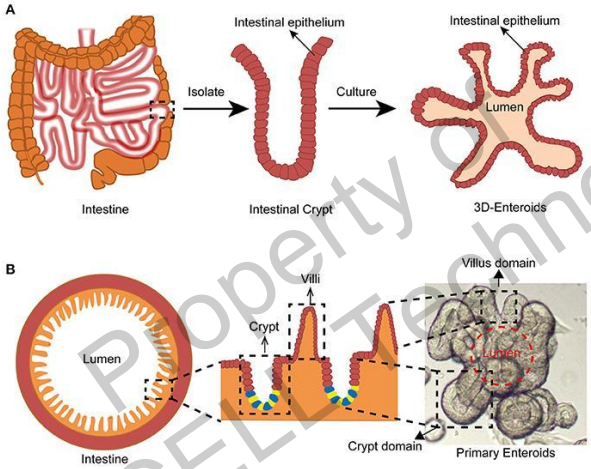
Immortalized cell line culture (Caco-2)



Advantages
- Cheap and easy
- Accessible apical surface

Disadvantage
- Not very representative

3D organoid culture

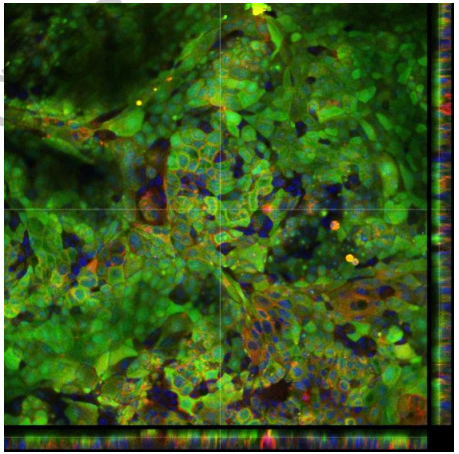


Advantages
- More physiologically relevant
- Scalable

Disadvantage
- Requires extracellular matrix

Adapted from Yin and Zhou (2018), Frontiers in Cellular and Infection Microbiology

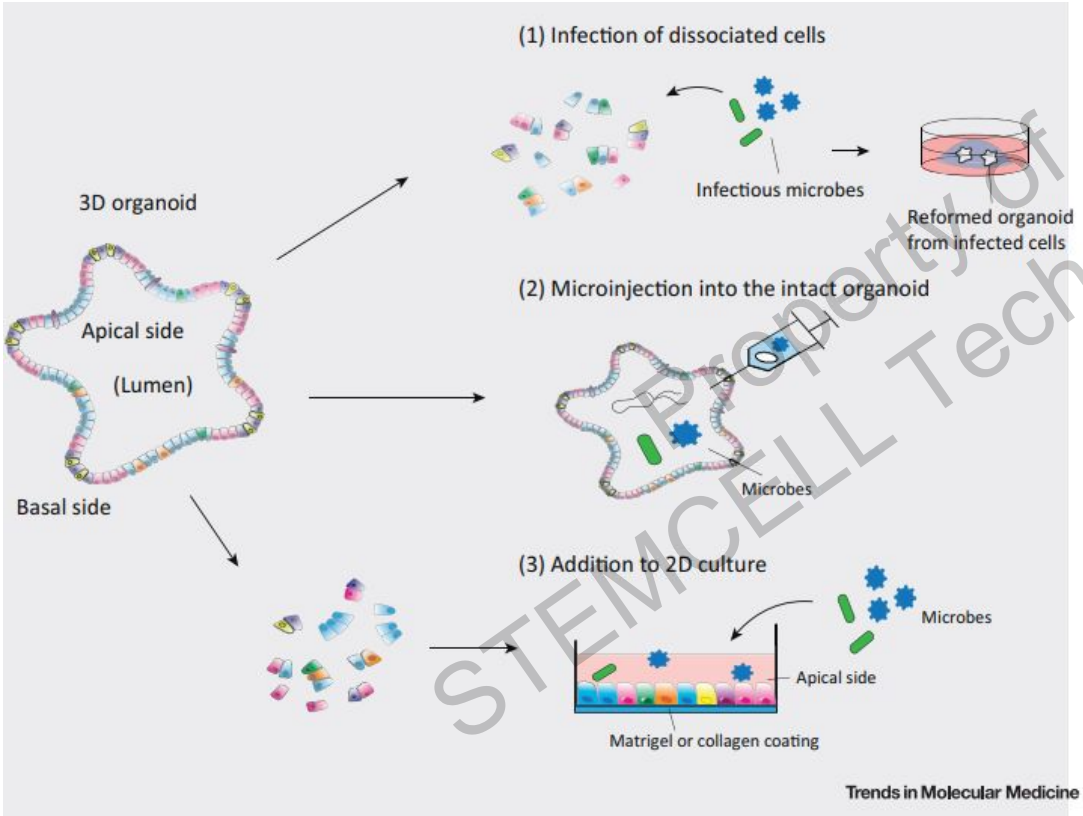
2D organoid-derived monolayer culture



Advantages
- Combines relevance and 2D
- Accessible apical surface

Disadvantage
- Lose 3D physiology

Intestinal Monolayers/ALI Cultures Are More Amenable for Infectious Studies



- Technically the easiest
- Difficult to follow infection in real time
- Limited in how host-pathogen interaction can be studied

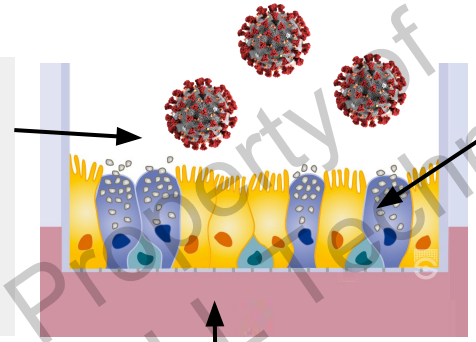
- Technically possible
- Laborious
- Not amenable for high-throughput applications

- Easy access to the apical side
- Good differentiation
- More amenable for high-throughput applications

Adapted from Dutta D. et al (2017), Trends in Molecular Medicine

Intestinal Monolayers/ALI: Evaluation of Viral Infection Model

Virus inoculation



Information from apical side:
- Morphological evaluation
- TEER measurement
- ELISA for apical cytokine analysis
- Barrier function/permeability assays

Information from tissue:
- Intracellular viral count
- Viral spread
- ICC/histology
- Ion channel activity assay
- Signaling pathways studies
- Innate immune responses

Information from basolateral side:
- Cytokine release
- Immune cell co-cultures

Summary

- The intestinal epithelium is primarily composed of six cell types arranged in a crypt-villus structure.
- Intestinal organoids provide a 3D in vitro intestinal model system that incorporates many of the physiologically relevant features of in vivo intestinal tissue. These features include a polarized epithelial layer surrounding a functional lumen.
- Primary intestinal organoids can be generated by isolating intestinal crypts that contain adult stem cells.
- In 2009, and 2011, Sato et al (Hans Clevers lab) published papers describing how to grow intestinal organoids from intestinal crypts isolated from human and mouse.
- Several alternative model systems are commonly used to model the intestine for a variety of applications. The most prevalent include in vivo studies using mouse models and intestinal cells lines such as Caco-2.
- Organoid derived intestinal-monolayer cultures can be a valuable model for applications requiring access to apical surface.