Development and Implementation of Human Intestinal Organoid Models for GI Toxicity

Julia Co, PhD

Sr. Principal Scientist, Complex *in vitro* Systems (CiS)

Safety Assessment, Genentech

COMPLEX IN VITRO SYSTEMS

Genentech
A Member of the Roche Group

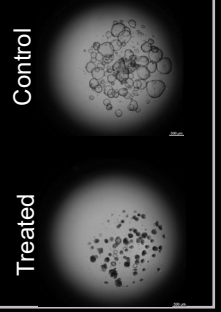
Goals for Intestinal *in vitro* Models

Goals

1. Enable modeling human GI biology with user-defined levels of complexity
2. Replace existing models (Caco-2, MDCK)
3. Reduce and sometimes replace animal models
4. Inform pre-clinical species selection

Safety Assessment

- De-risking and predicting GI tox
- Mechanisms of Tox
- Understanding GI tox in the clinic
- Stratifying patient populations/understanding patient diversity



Biomarkers

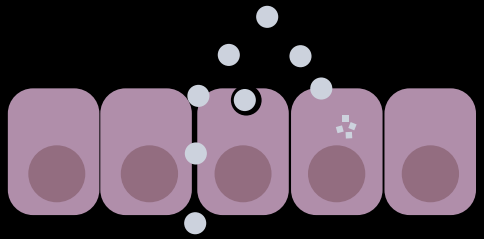
- Identify novel biomarkers to determine target engagement and disease progression in the clinic

Disease Modeling and Discovery

- Determine mechanisms of action/pathogenesis
- Target discovery
- Screen libraries in a relevant GI model

ADME

- Drug uptake/transport/bioavailability
- Drug metabolism

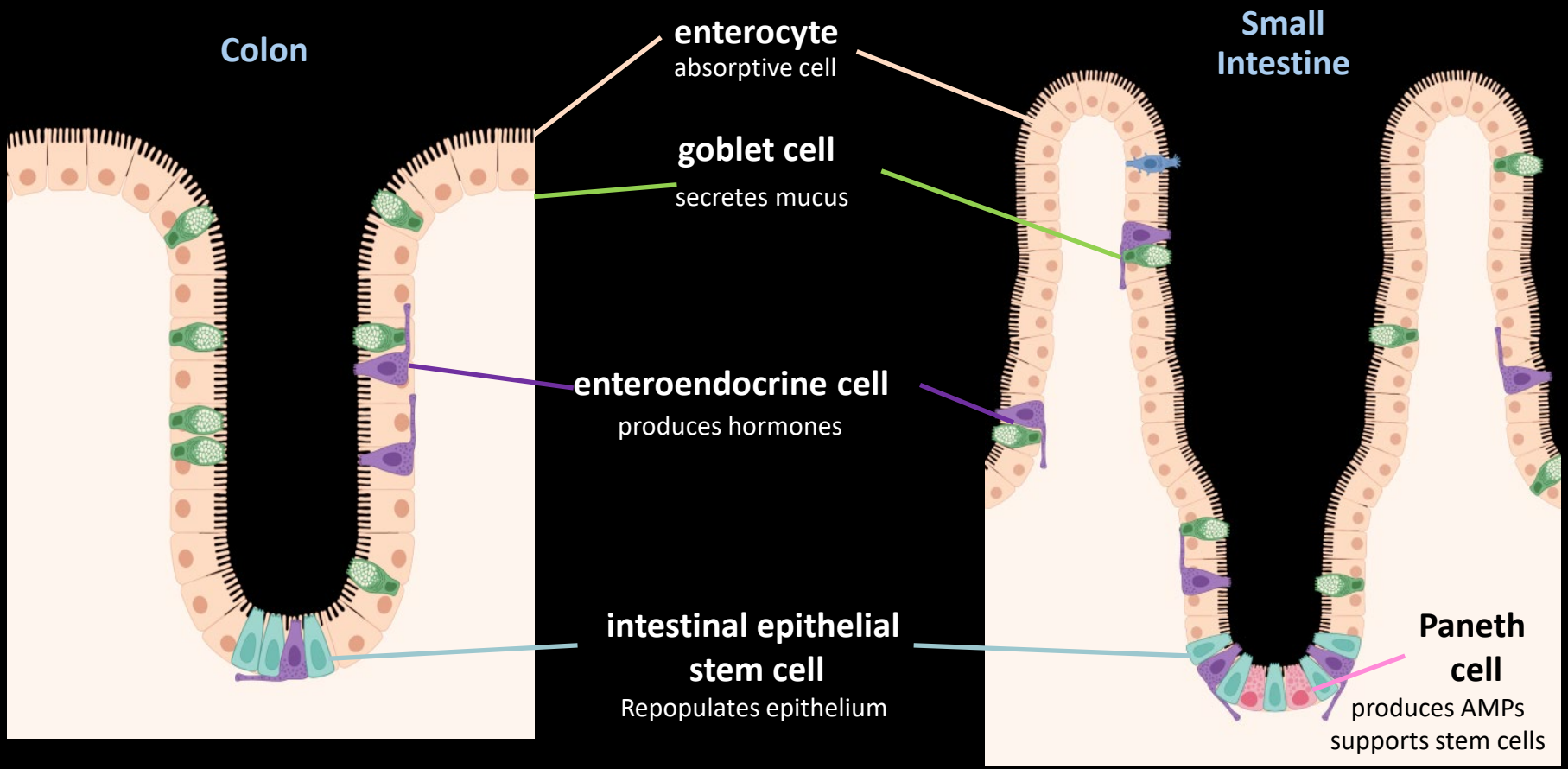


D&I

- Diversity of biological responses to molecules/compounds

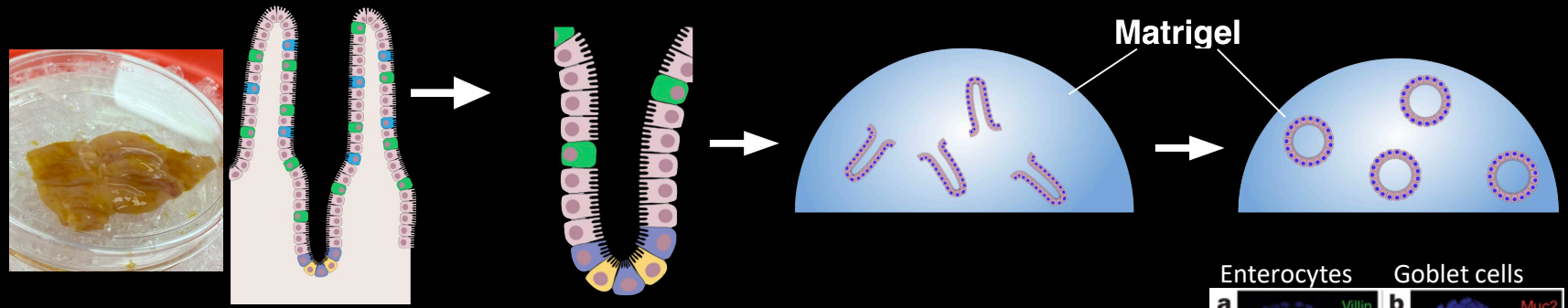
Genentech
A Member of the Roche Group

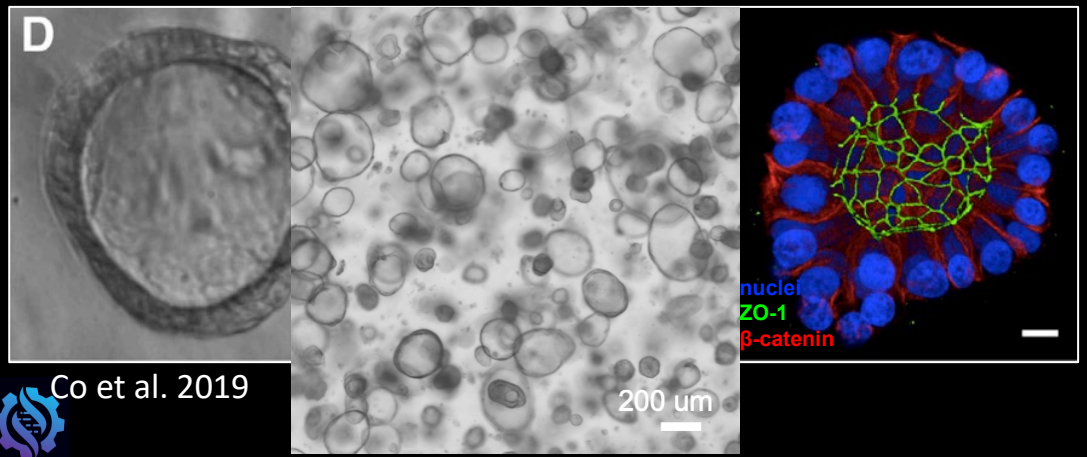
Intestinal Epithelial Cells



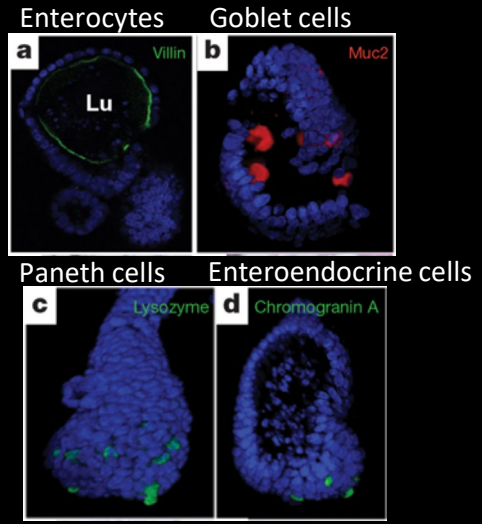
Created with Biorender.com

Human Primary Tissue-Derived Intestinal Epithelial Organoids





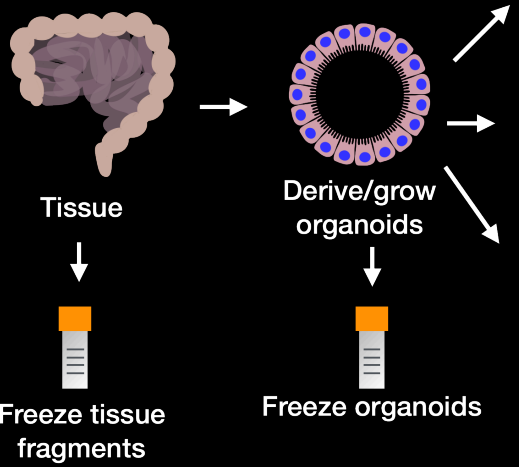
Co et al. 2019



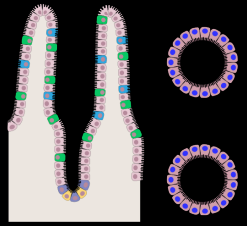
Sato et al. 2009

Intestinal *in vitro* Models

ORGANOID CULTURE



ORGANOID-DERIVED MODELS



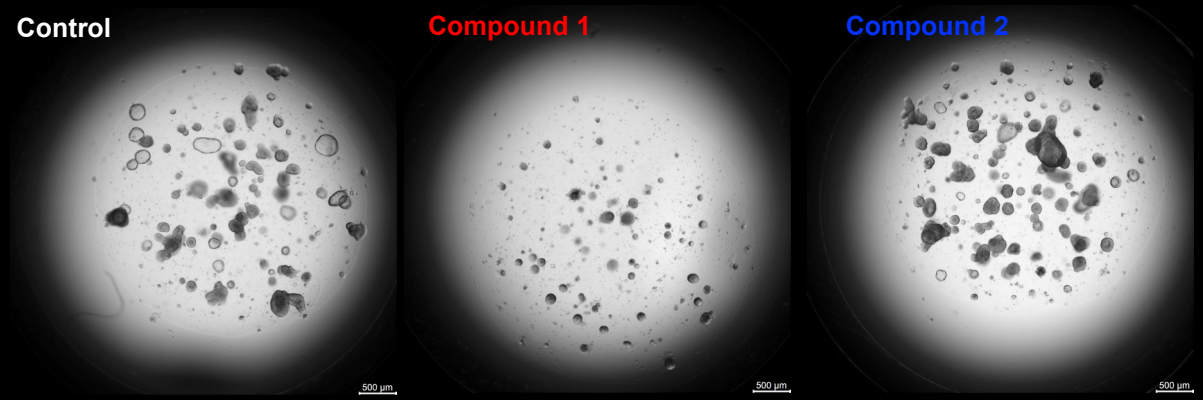
Organoid spheroids

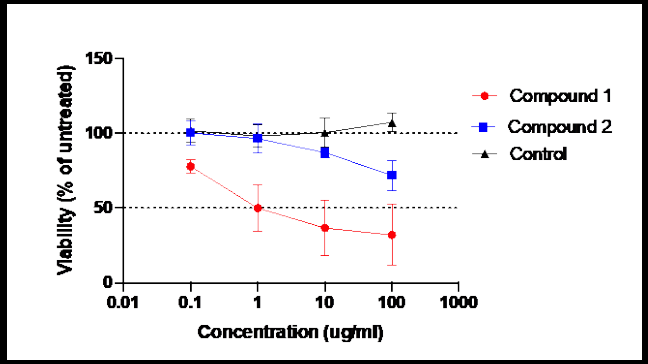
Stem cell organoids (crypt-like)

Differentiated organoids (villus-like)

Genentech
A Member of the Roche Group

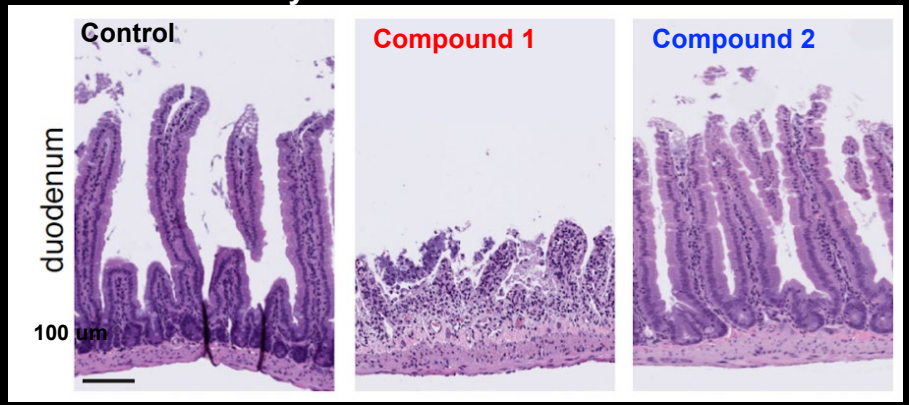
Organoids for Predictive GI Toxicity Studies





n=4 plates in 3 independent experiments , Error bars are S.D.
Viability determined by CellTiter Glo 3D ATP assay

***in vivo* mouse study**

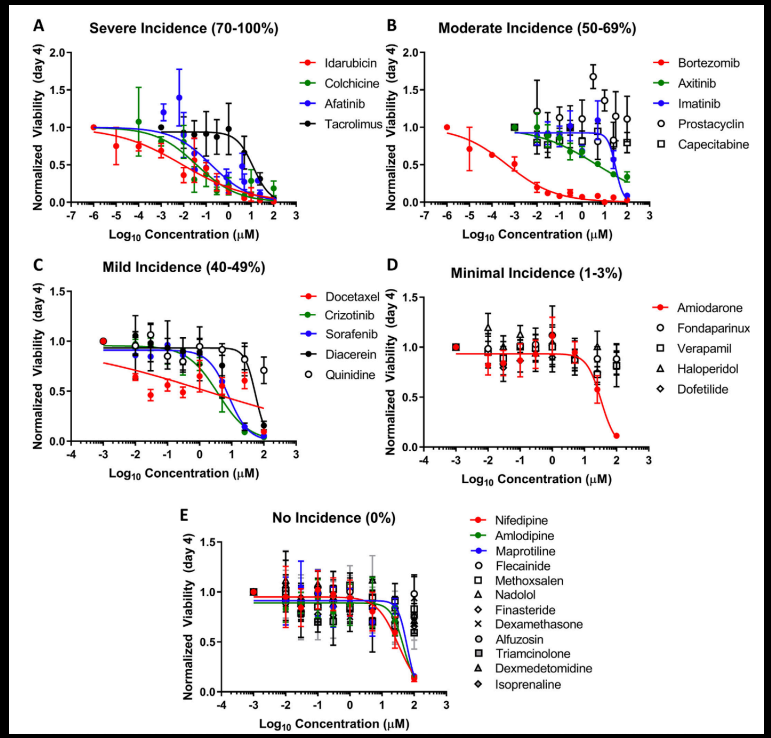


Organoids facilitate lead optimization and reduction in scale of animal studies

Felipe de Sousa e Melo, JT Koerber, Catherine Ruff, Melissa Schutten, Nina Ljumanovic, Michelle Lepherd, Donna Lee

Genentech
A Member of the Roche Group

Intestinal Organoid Models for Safety Assessment



Belair et al. Tox in vitro (2020)

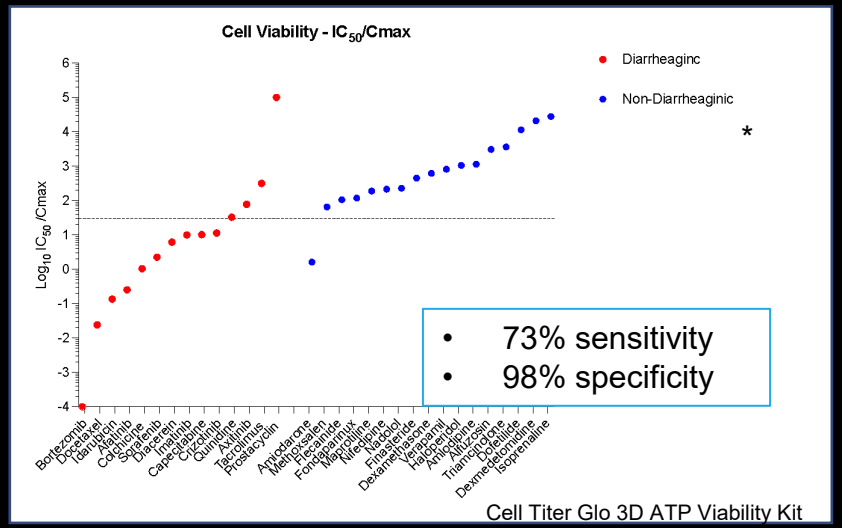

Jessica Klein


Julia Heidmann


Lanlan Yu

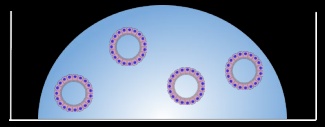

Tomo Kiyota

Validating Context-of-Use Assays

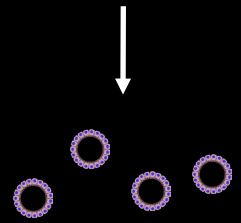


*where no IC_{50} was achievable, maximum concentration tested was used to calculate ratio
Compound list derived from Belair et al. Toxicol In Vitro 2020

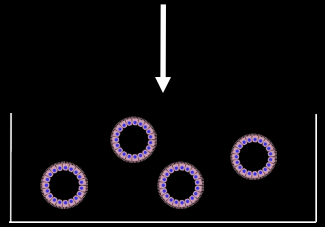
Organoid Polarity can be Reversed for Apical Access



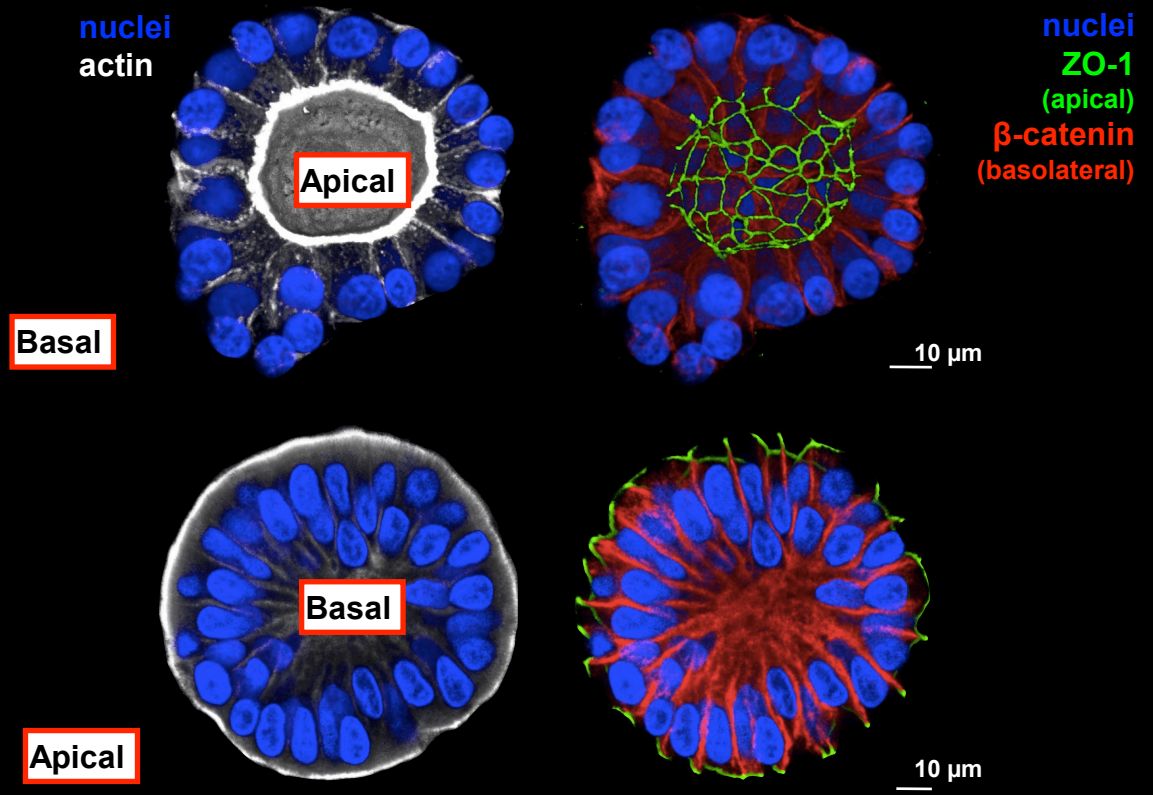
Grow organoids submerged in Matrigel



Dissociate ECM to isolate organoids



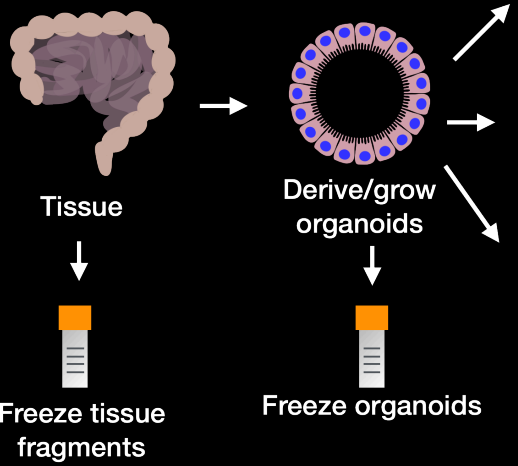
Suspend organoids in growth media



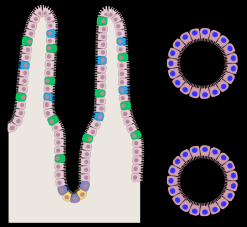
Co et al., *Cell Reports* (2019); Co* et al. *Nature Protocols* (2022)

Intestinal *in vitro* Models

ORGANOID CULTURE



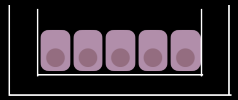
ORGANOID-DERIVED MODELS



Stem cell organoids (crypt-like)

Differentiated organoids (villus-like)

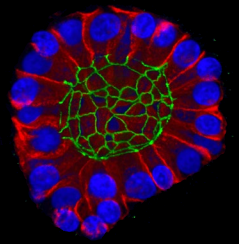
Organoid spheroids

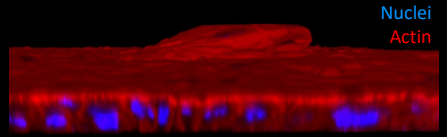


Stem cell monolayers

Differentiated monolayers

Transwell monolayers



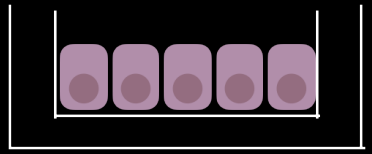


Genentech
A Member of the Roche Group

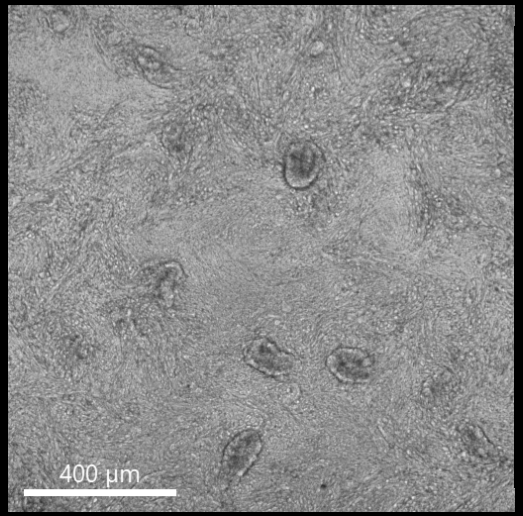
GI Organoid-Derived Transwell Monolayers

Cultivate organoids in matrix

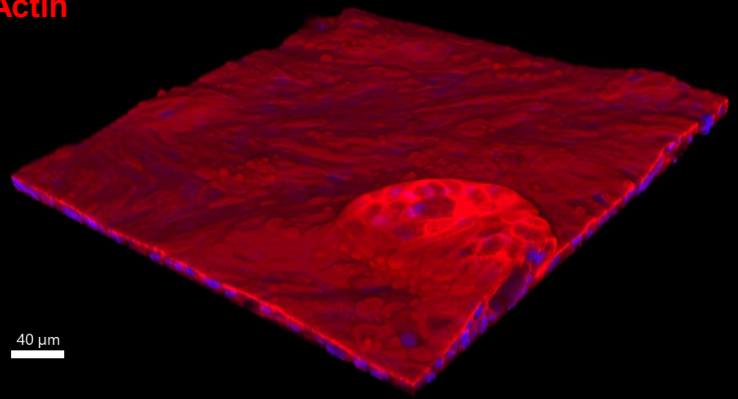
Dissociate to single cells with TrypLE Express



Seed on matrix-coated transwell membrane



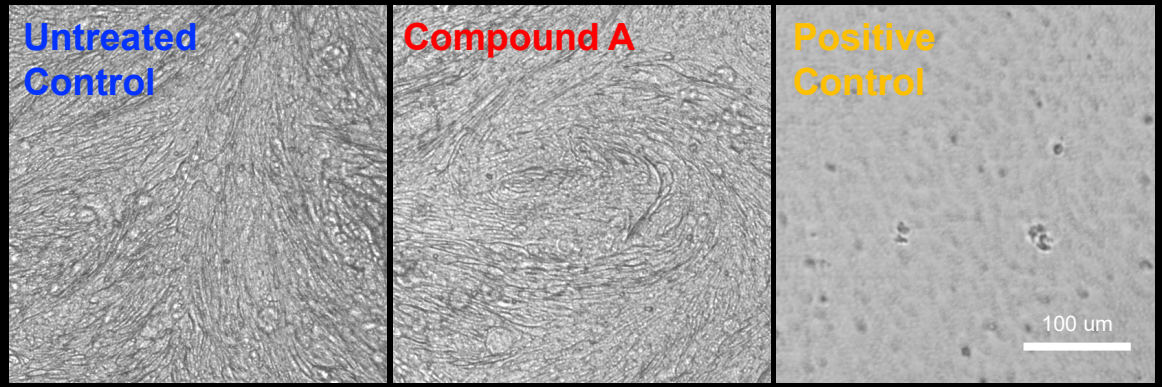
Nuclei
Actin

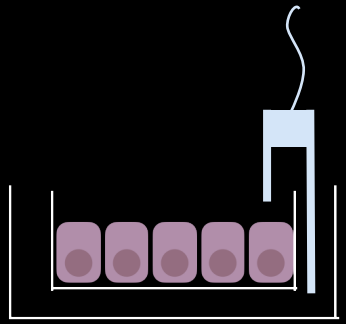


- Requires a lot of cells
- Enables apical/basolateral access
- Compatible with kinetic barrier function measurements

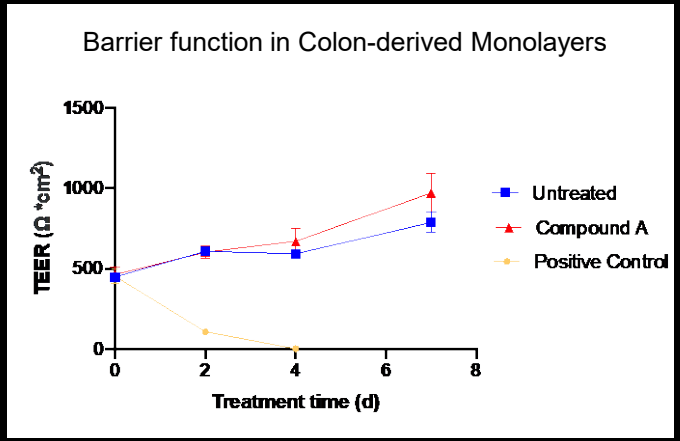
Genentech
A Member of the Roche Group

Organoid-Derived Transwell Monolayers for GI Toxicity





Trans-Epithelial Electrical Resistance (TEER) as a measurement of barrier function



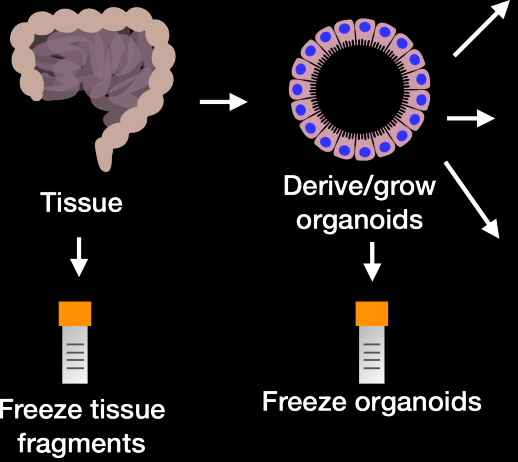
n = 4 replicates, Error bars represent S.D.

Organoid models enable compound evaluation in conditions not achievable *in vivo*

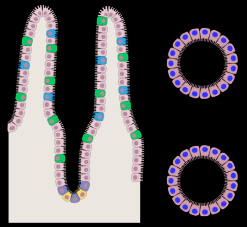
Genentech
A Member of the Roche Group

Intestinal *in vitro* Models

ORGANOID CULTURE



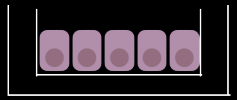
ORGANOID-DERIVED MODELS



Stem cell organoids (crypt-like)

Differentiated organoids (villus-like)

Organoid spheroids



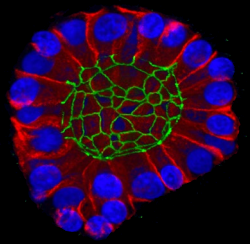
Stem cell monolayers

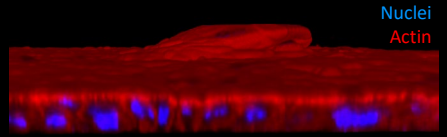
Differentiated monolayers

Transwell monolayers

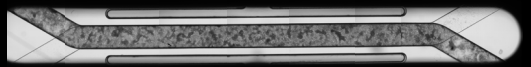
Differentiated MPS (+/- stretch)

MPS chip





Emulate Colon Chip



Genentech
A Member of the Roche Group

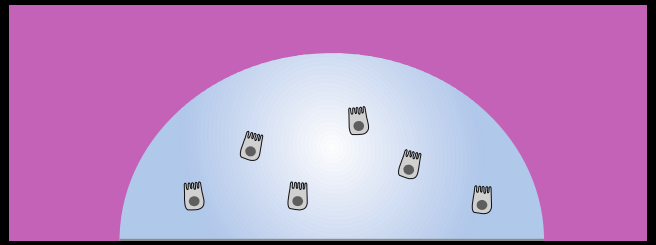
Model Selection Considerations

- Scale per sample
- Scale – number of samples
- Apical/basal epithelium access
- Mechanical properties (fluid flow, stretch, etc.)
- Co-culture compatibility
- Multi-organ linkage
- Readout compatibility
    - Assay type
    - Live vs endpoint
    - Sample collection volume

How

Existing Organoid Culture Method

Attached BME Dome Cultue

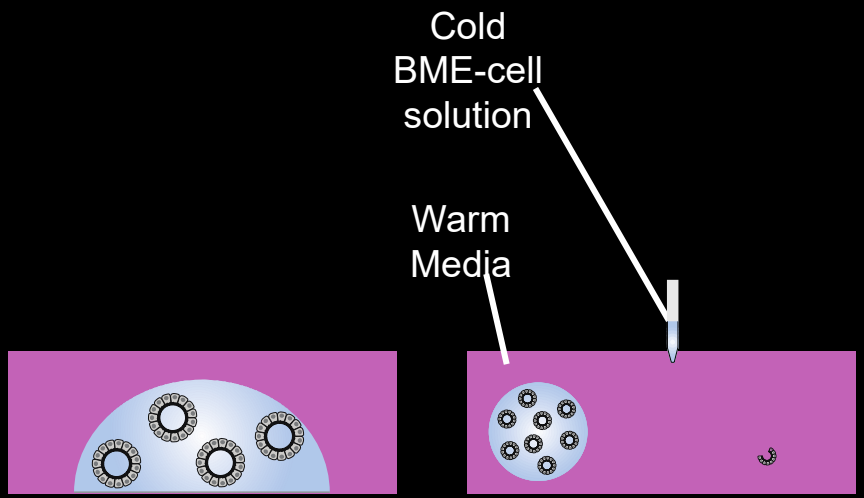


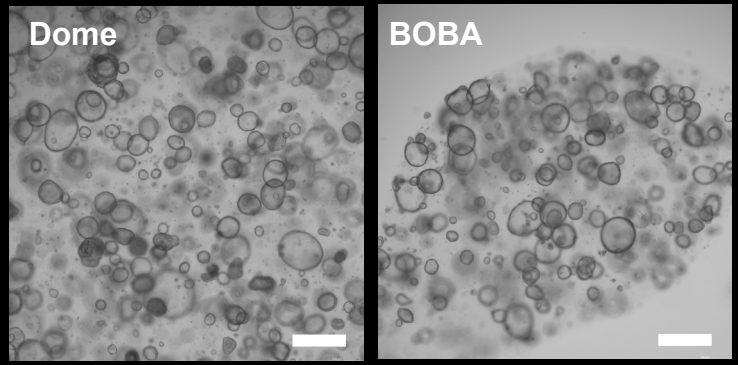
1. Deposit cells + cold BME/Matrigel (liquid) in center of well.
2. Incubate at 37C to cure BME/Matrigel.
3. Add media to each well.

SigmaAldrich.com

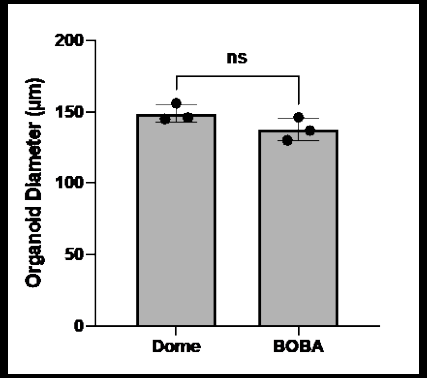
Genentech
A Member of the Roche Group

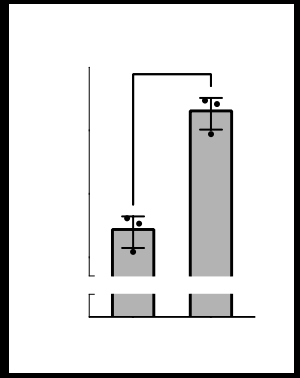
Suspended BME Hydrogel Organoid Culture





BOBA
(BME-embedded
Organoid Bead
Assemblies)

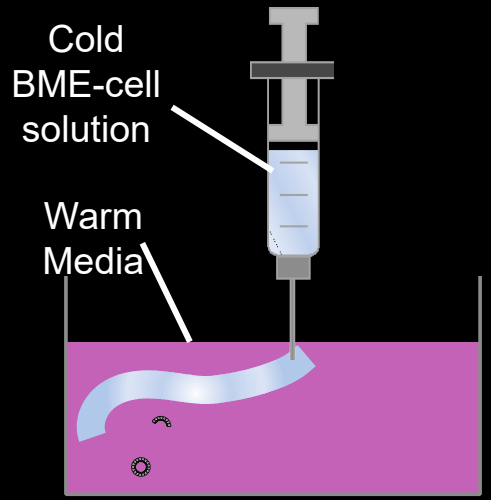




Genentech
A Member of the Roche Group

Co et al., *Sci Rep* (2023)

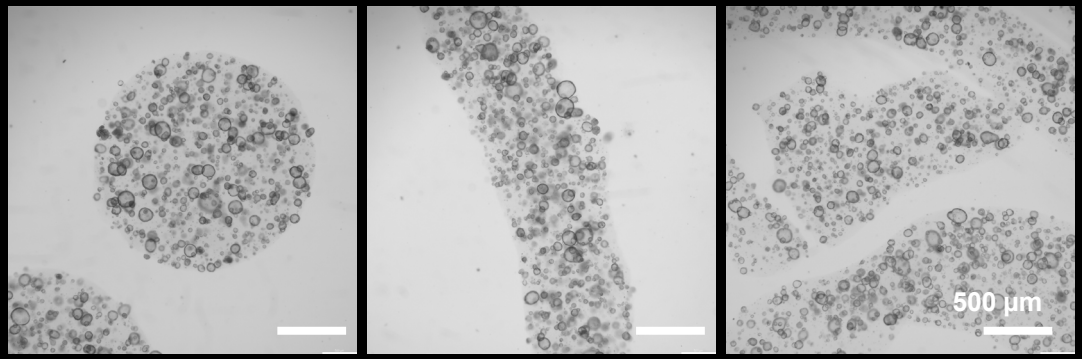
Suspended BME Hydrogel Organoid Culture

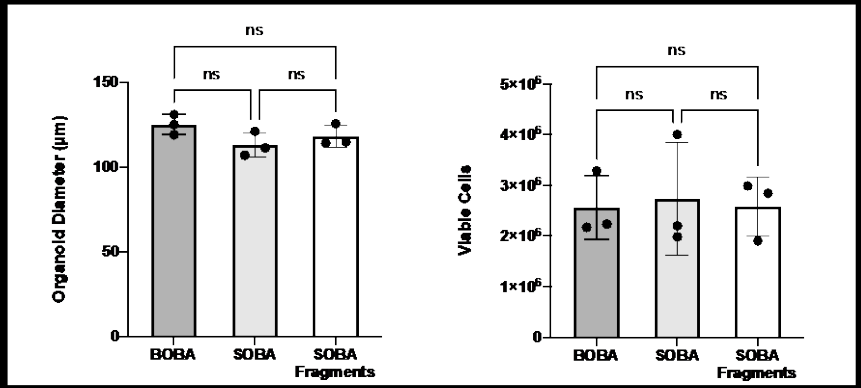


Suspended Filaments

SOBA
(Syringe-extruded Organoid BME Assemblies)

BOBA (droplets) **SOBA (filaments)** **SOBA Fragments**

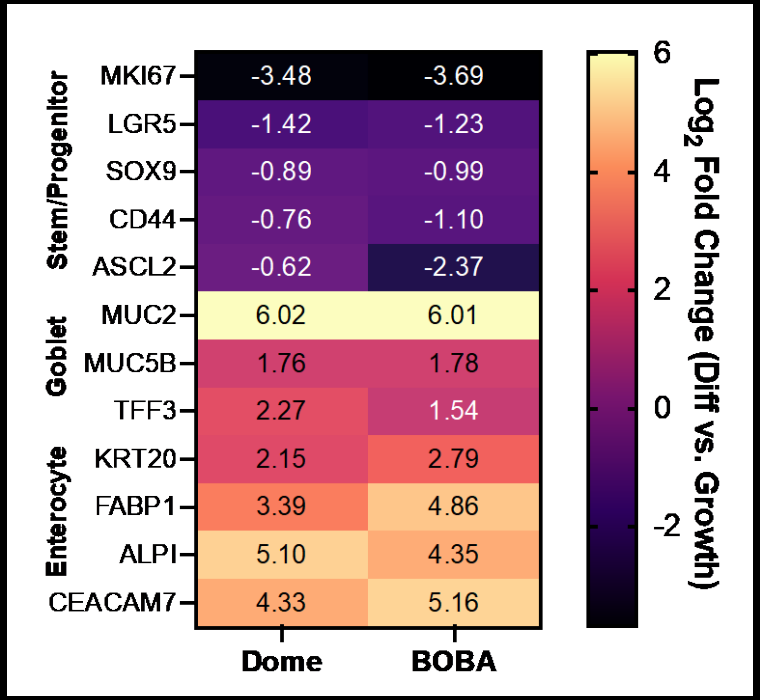


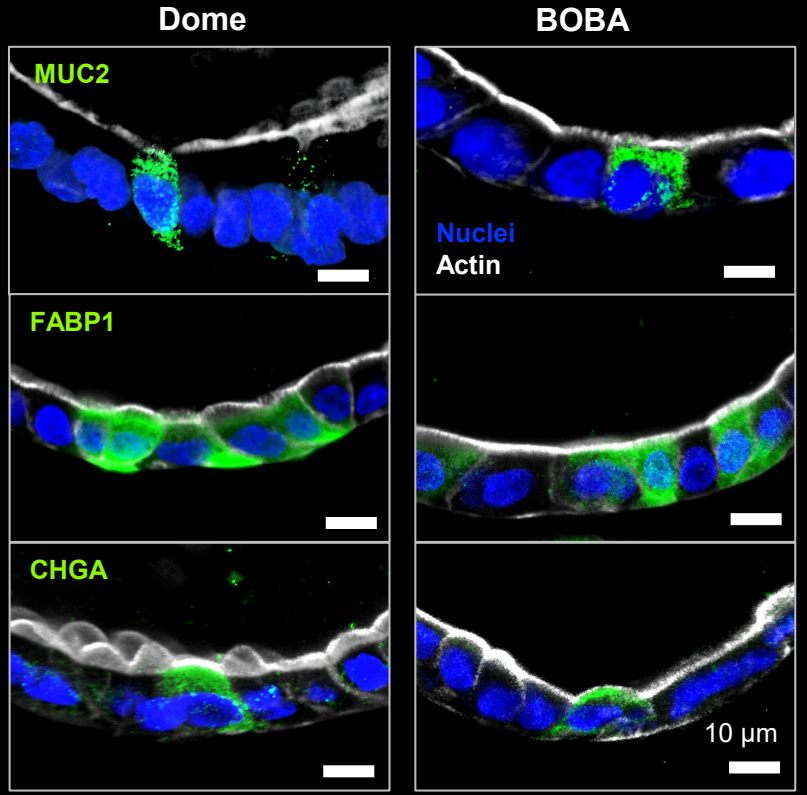


Genentech
A Member of the Roche Group

Co et al., *Sci Rep* (2023)

Organoid differentiation in Dome and BOBA culture is comparable



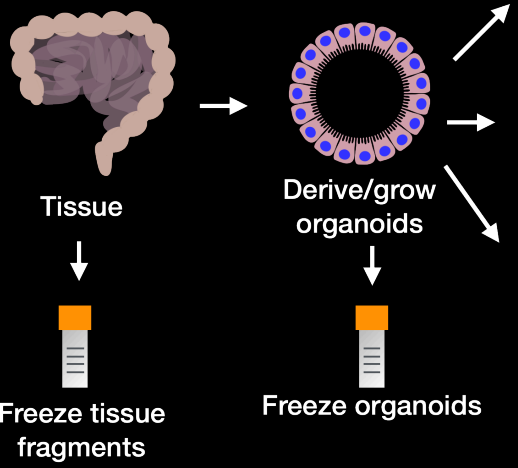


Co et al., *Sci Rep* (2023)

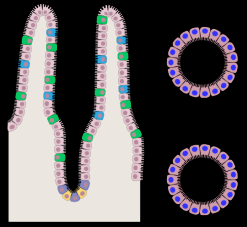
Genentech
A Member of the Roche Group

Intestinal *in vitro* Models

ORGANOID CULTURE



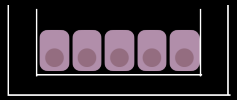
ORGANOID-DERIVED MODELS



Stem cell organoids (crypt-like)

Differentiated organoids (villus-like)

Organoid spheroids



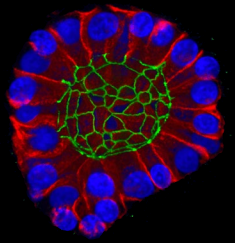
Stem cell monolayers

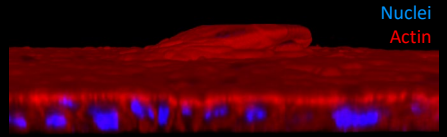
Differentiated monolayers

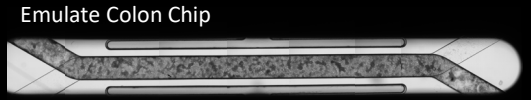
Transwell monolayers

Differentiated MPS (+/- stretch)

MPS chip







Genentech
A Member of the Roche Group

Next Steps for the field: Implementation of GI NAMs for Drug Development

- Confidence in the models
  - Comparative characterization of NAMs and physiologic tissue – omics and functional assays
  - Inform model selection for each application

- Confidence in the assays
  - Qualification of context-of-use assays comparing in vitro to clinical outcomes
  - Protocol standardization

- Building large datasets
  - Shared and accessible data

- Using NAMs to model human diversity
  - Accessibility to organoid cells from diverse donors

Genentech
A Member of the Roche Group

Acknowledgements

Complex in vitro Systems
Kim Homan
Jessica Klein
Serah Kang
Sarah Madira
Elias Kahn

Investigative Toxicology
Aaron Fullerton
Tomo Kiyota
Julia Heidmann
Lanlan Yu

Toxicology
Donna Lee
Catherine Ruff
Michelle Lepherd
Nina Ljumanovic

SA Pathology
Catherine Ruff
Michelle Lepherd

PD PBS
Vidhyalakshmi Arumugam
Sugantha Balasubbu
Melanie Dela Cruz
Aayushi Trivedi

Felipe de Sousa e Melo
JT Koerber
Mary Keir
Loryn Holokai

Genentech
A Member of the Roche Group