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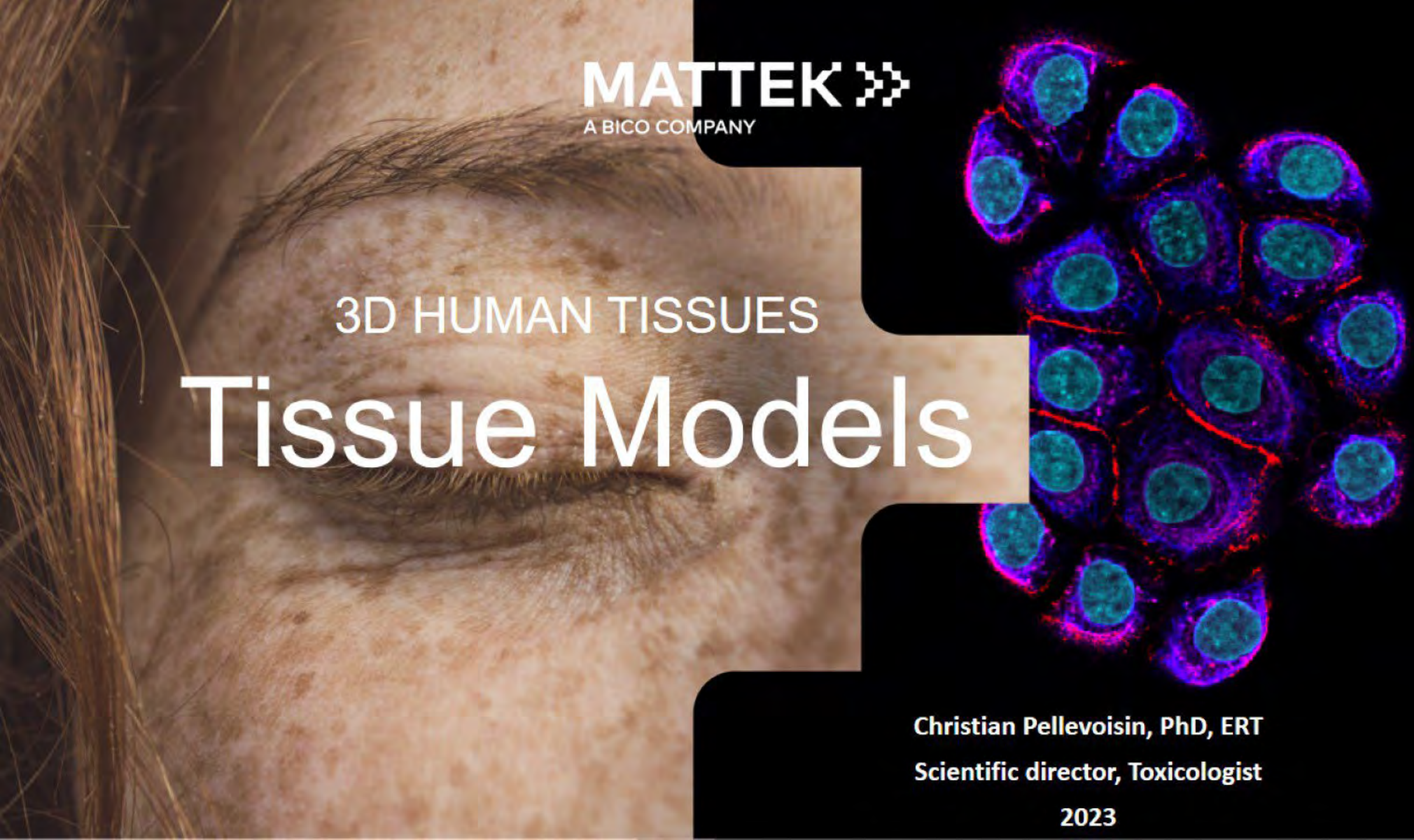


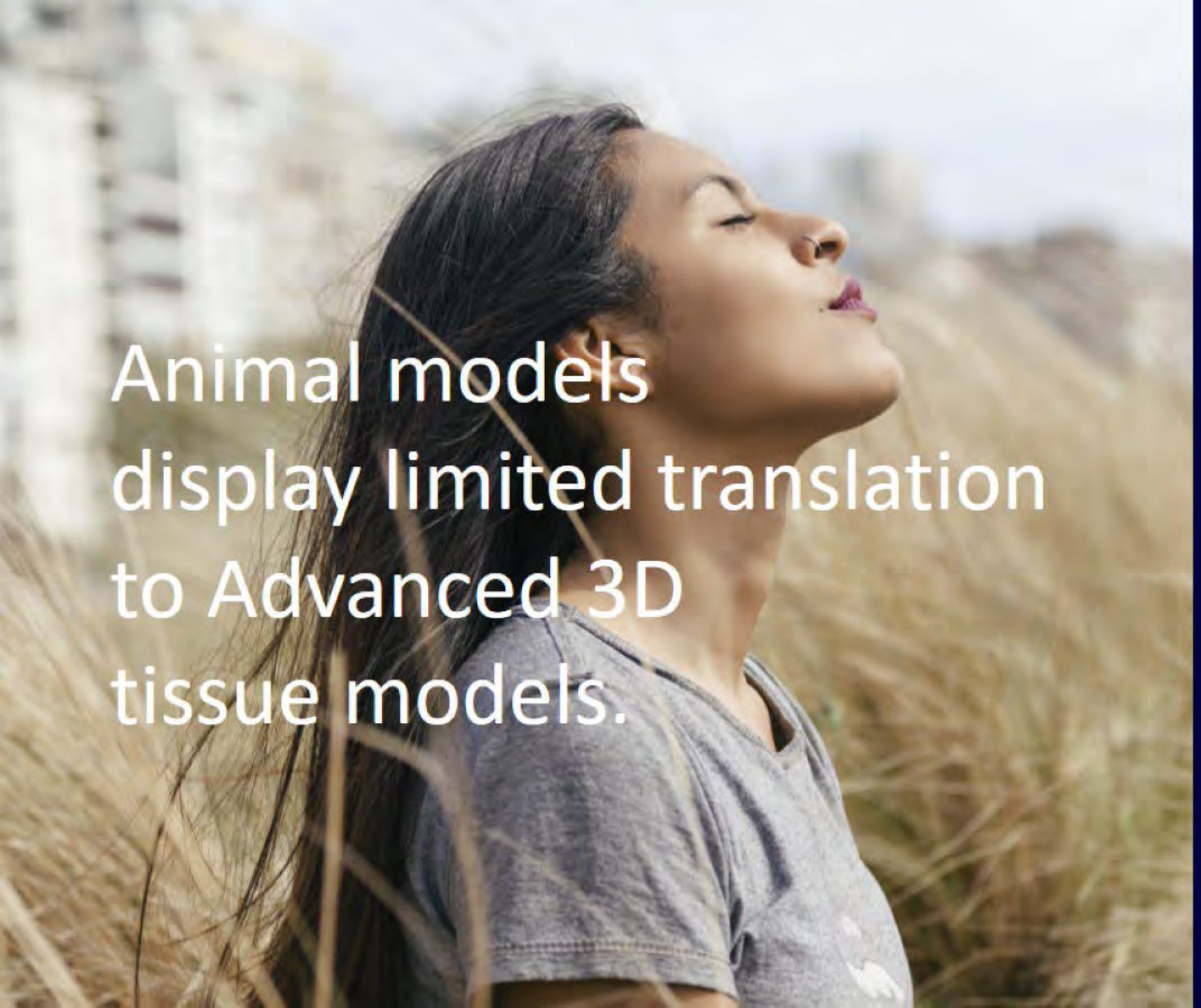
3D HUMAN TISSUES

Tissue Models

Christian Pellevoisin, PhD, ERT

Scientific director, Toxicologist

2023

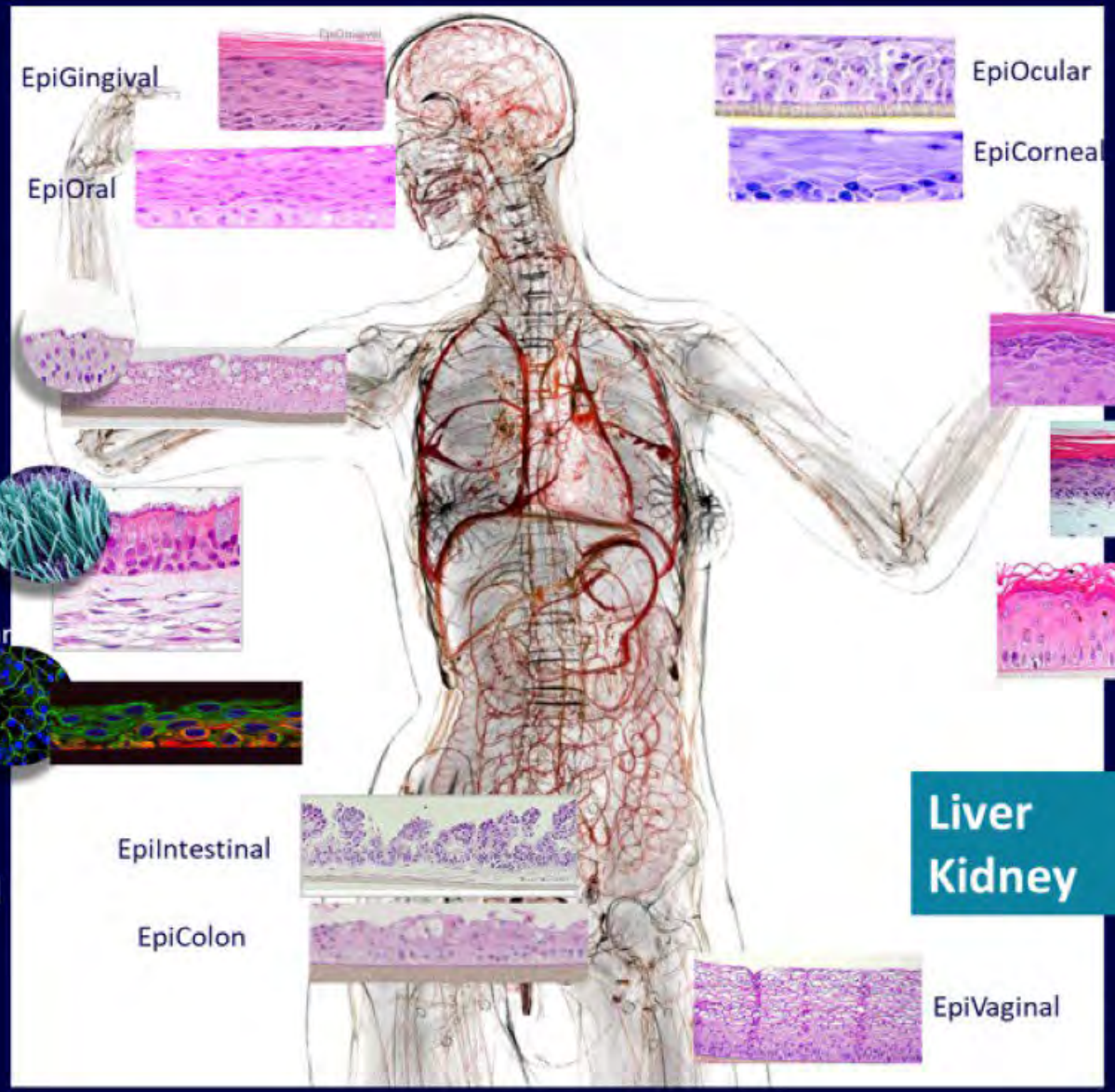


Animal models display limited translation to Advanced 3D tissue models.

Continued data demonstrates that results from animal studies do not always translate to humans

Animal models cannot fully replicate genetic, molecular, immunologic, and cellular responses to humans

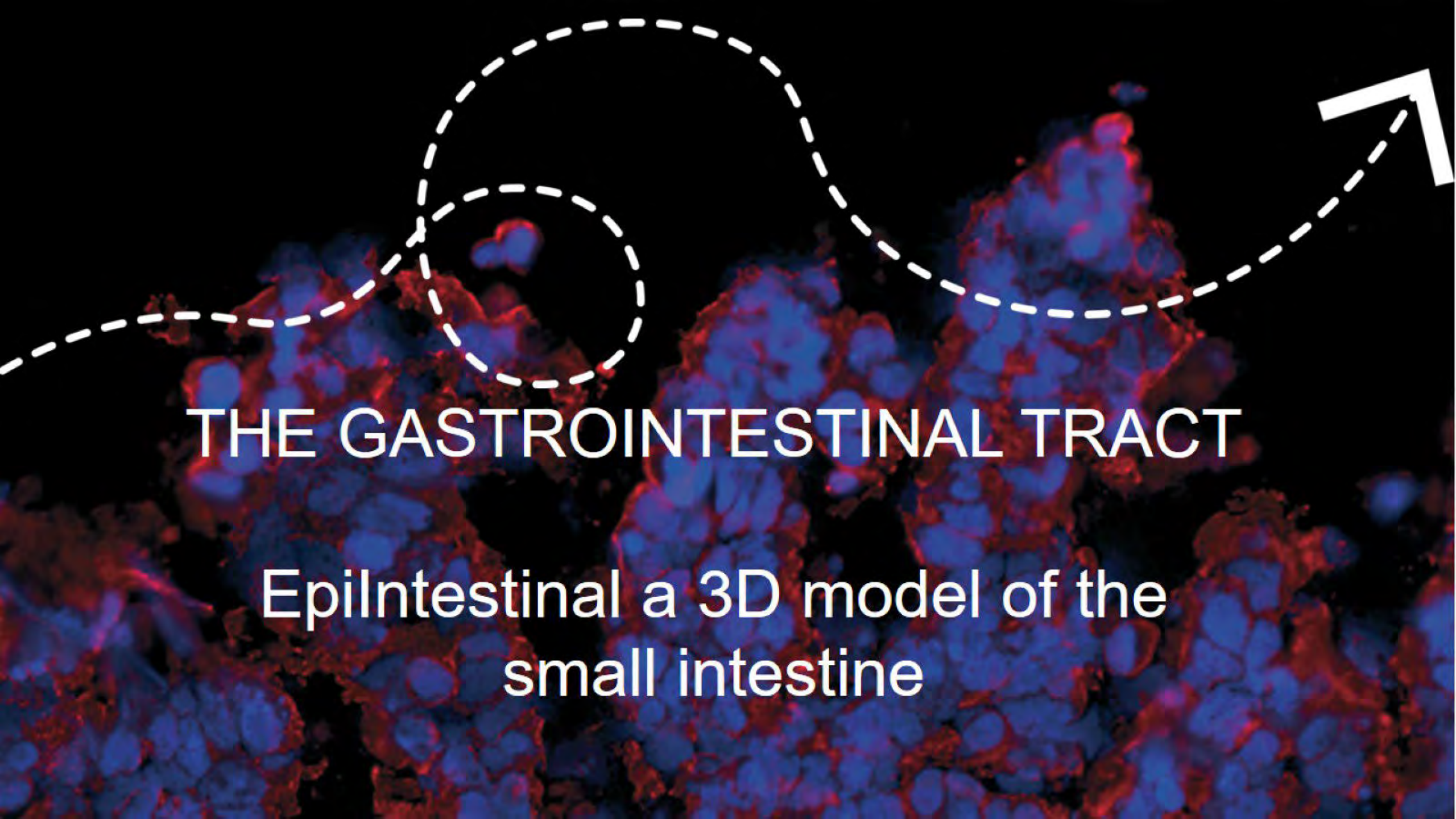
Animal models are expensive, time-intensive, have inter-species extrapolation issues, and are low-throughput



https://bryanchristiedesign.com/woman-figure

INDUSTRIAL TISSUE PRODUCTION

https://www.youtube.com/watch?v=3vpJV1XDij8


THE GASTROINTESTINAL TRACT
EpiIntestinal a 3D model of the small intestine

Air-liquid Interface (ALI) Technology

Cell Isolation –
Epithelial Cells
Fibroblasts

Cell Expansion

Cells are
Cryopreserved

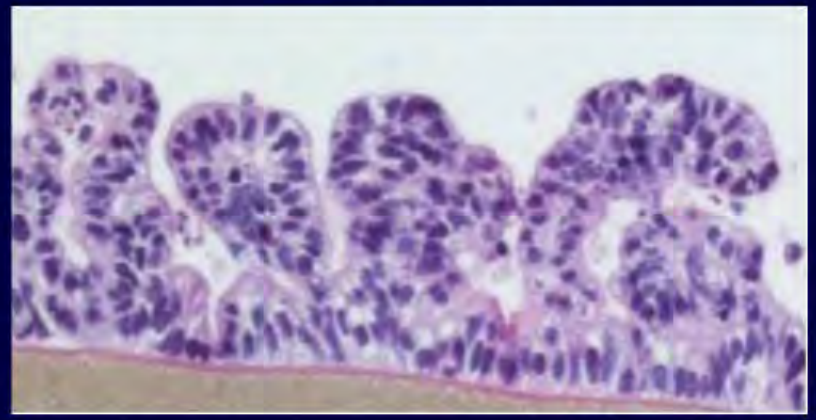
Cells Seeded onto
Tissue Culture
Inserts

Tissue
Differentiation &
Stratification

Fully
Differentiated
Tissue

EpiIntestinal

MatTek's 3D tissue model of the human small intestine is our latest innovation advancing in vitro GI research worldwide. Allowing for physiological exposures and human-relevant endpoints, EpiIntestinal is being incorporated into pharmaceutical development programs across the globe.



GI TOXICITY

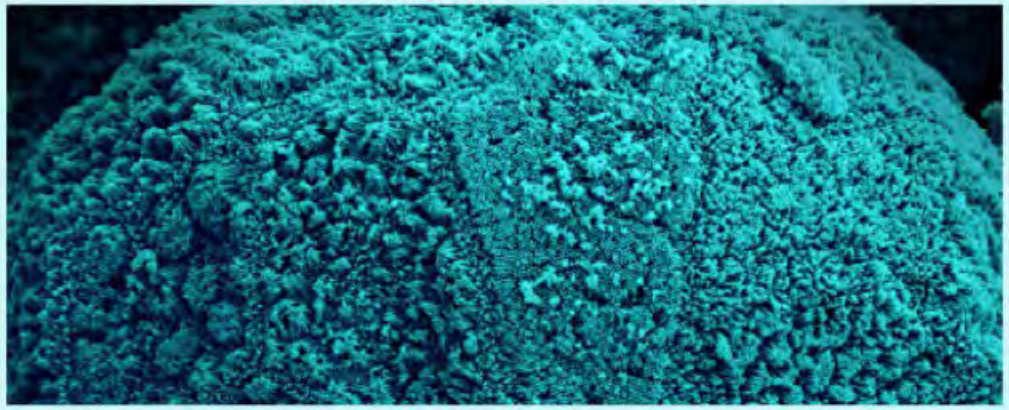
GI INFECTION

DRUG METABOLISM

DRUG DELIVERY

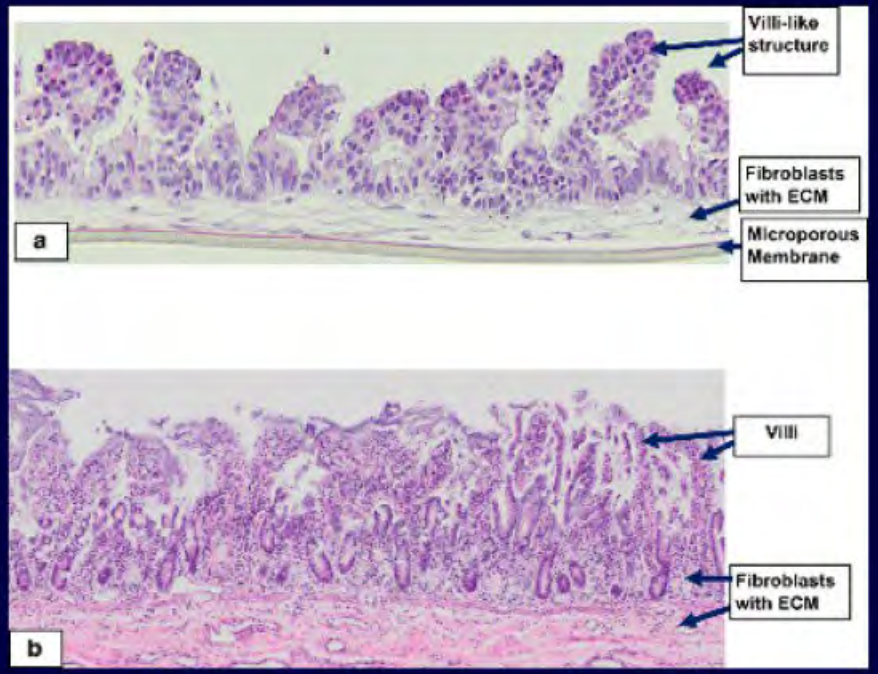
INFLAMMATION AND FIBROSIS

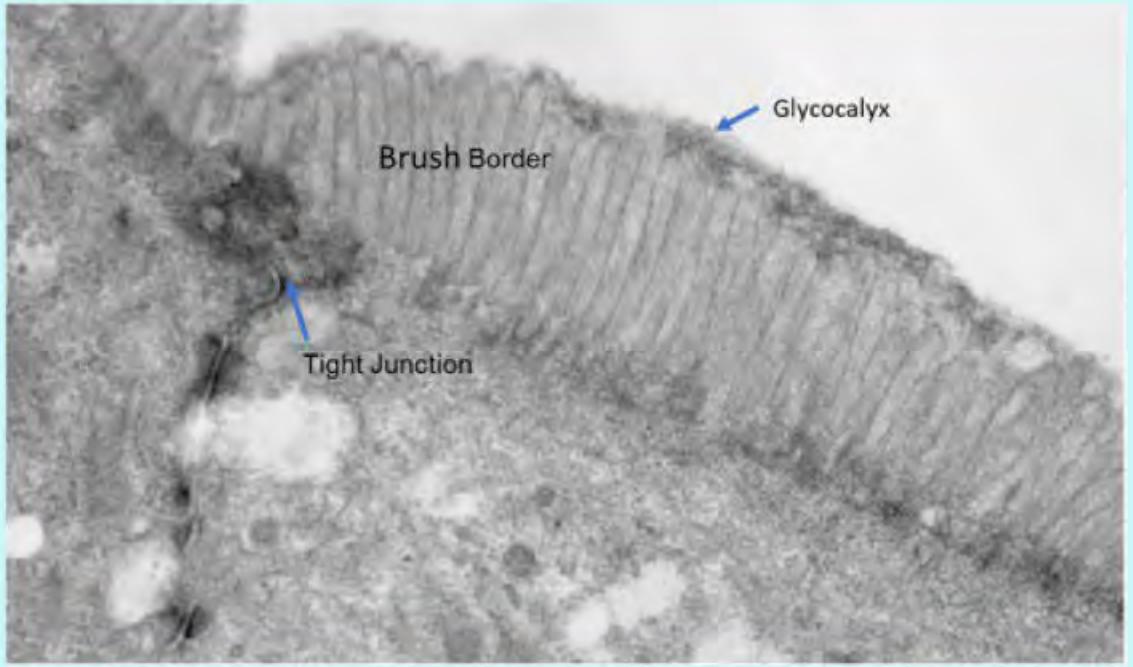
WOUND REPAIR



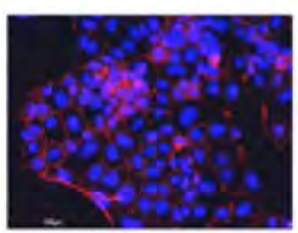
A human 3D in vitro small intestinal tissue model with epithelial polarity to evaluate toxicity, metabolism, drug absorption and compound efficacy. MatTek's latest novel innovation to help redefine preclinical testing.

H&E stained histological cross-section of the full-thickness **EpiIntestinal tissue model (*a*)** and the **in vivo explant small intestine (*b*)** showing the apical epithelium with villi structure and the underline structure of fibroblast-containing collagen matrix. Note: The EpiIntestinal tissue was grown on an underlying microporous membrane (pore diameter = 0.4 µm)

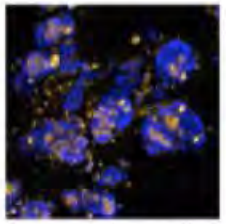




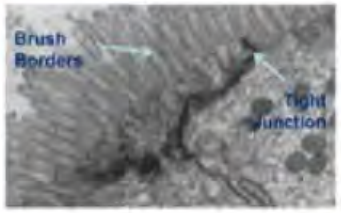
Human small intestinal organotypic culture model for drug permeation, inflammation, and toxicity assays Jan Markus et al., 2020, https://doi.



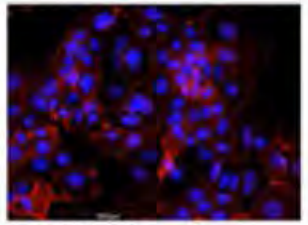
Tight junctions
Claudin 1



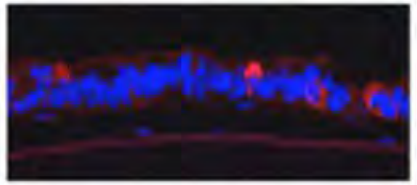
Tuft Cells
DCLK1 (Doublecortin-Like Kinase 1)



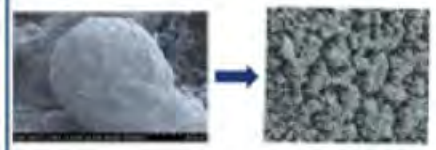
TEM microphotograph



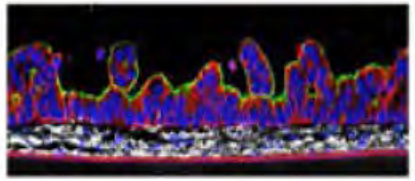
Tight junctions
ZO-1



Goblet Cells/mucus
MUC-2



SEM microphotograph
Villi-like structure + brush border detail



Villin, CK19, Vimentin

Paneth cells
Lysozyme

Stem Cells
Lgr5

Morphology and Cellular Markers of EpiIntestinal

Left and Middle Column: IHC staining of various proteins characteristic for different cell types or structures. Nuclei are shown blue.

Right Column: Transmission and scanning electron microscopy used to visualize villi, brush borders, and tight junctions;

Bottom: IHC staining for Lgr5 – a marker of stem cells.

EpiIntestinal permeability compared to Caco-2 cells

- Caco-2 and EpiIntestinal exposed to low (Talinolol), moderate (Ranitidine), and high (warfarin) absorption drugs in humans
- Results compared to historical absorption data from humans
  - Caco-2 unable to distinguish between low and moderately absorbed compounds while EpiIntestinal successfully discriminated

Table V Comparison of Drug Permeation in the SMI Microtissues and the Caco-2 Cell Monolayers for Three Model Drugs: 1) Talinolol = Low Permeability Drug, 2) Ranitidine = Moderate Permeability Drug but Poorly Absorbed in Caco-2 Cell Monolayers, c) Warfarin = High Permeability Drug

	In vitro		*In vivo*
	P_{app} A- > B (10^{-6} cm^{s-1})		
Test compounds	SMI microtissues	Caco-2 cells	Fraction absorbed (%) in humans
Talinolol	0.8 ± 0.1	0.2 ± 0.2	54
Ranitidine	2.2 ± 0.5	0.2 ± 0.0	61
Warfarin	22.3 ± 0.5	27.8 ± 7.6	100

Mean P_{app} values from n = 2 SMI microtissue lots are presented

Ayehunie et al. Human Primary Cell-Based Organotypic Microtissues for Modeling Small Intestinal Drug Absorption. Pharm Res (2018) 35:72.

pharmaceutics

MDPI

Article

In-Depth Characterization of EpiIntestinal Microtissue as a Model for Intestinal Drug Absorption and Metabolism in Human

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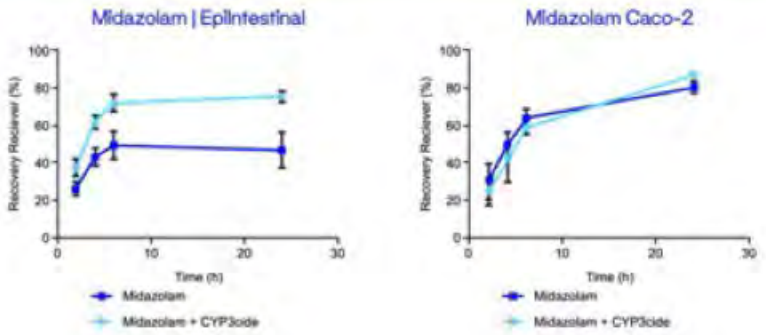
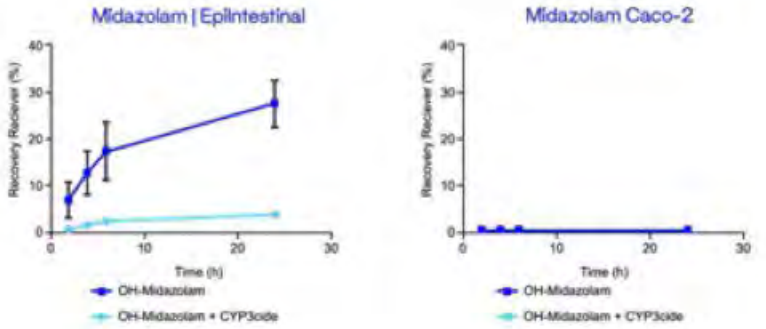
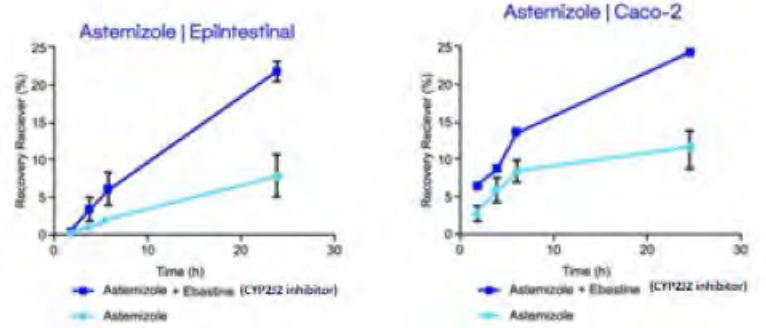
check for updates

Abstract: The Caco-2 model is a well-accepted in vitro model for the estimation of fraction absorbed in human intestine. Due to the lack of cytochrome P450 3A4 (CYP3A4) activities, Caco-2 model is not suitable for the investigation of intestinal first-pass metabolism. The purpose of this study is to evaluate a new human intestine model, EpiIntestinal microtissues, as a tool for the prediction of oral absorption and metabolism of drugs in human intestine. The activities of relevant drug transporters and drug metabolizing enzymes, including MDR1 P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), CYP3A4, CYP2J2, UDP-glucuronosyltransferases (UGT), carboxylesterases (CES), etc., were detected in functional assays with selective substrates and inhibitors. Compared to Caco-2, EpiIntestinal microtissues proved to be a more holistic model for the investigation of drug absorption and metabolism in human gastrointestinal tract.







Cui et al. In-Depth Characterization of EpiIntestinal Microtissue as a Model for Intestinal Drug Absorption and Metabolism in Human. Pharmaceuticals 2020, 12, 405.

Metabolism –EpiIntestinal has improved CYP3A4 activity compared to Caco-2 cells

- Drug metabolizing enzyme studied with Midazolam (CYP3A4) and Astemizole (CYP2J2)
- CYP3A4 inhibitor CYP3cide had almost no effect on Caco-2 but increased availability of midazolam in EpiIntestinal
- High levels of 1-hydroxymidazolam, selective CYP3A4 metabolite of midazolam in EpiIntestinal but not Caco-2
- CYP2J2 readily detected in both models and inhibited by Ebastine

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TOXICOLOGICAL SCIENCES, 168(1), 2019, 3–17

doi: 10.1093/toxsci/kfy268
Advance Access Publication Date: October 26, 2018
Research Article

Human 3D Gastrointestinal Microtissue Barrier Function As a Predictor of Drug-Induced Diarrhea

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ABSTRACT

Drug-induced gastrointestinal toxicities (GITs) rank among the most common clinical side effects. Preclinical efforts to reduce incidence are limited by inadequate predictivity of *in vitro* assays. Recent breakthroughs in *in vitro* culture methods support intestinal stem cell maintenance and continual differentiation into the epithelial cell types resident in the intestine. These diverse cells self-assemble into microtissues with *in vivo*-like architecture. Here, we evaluate human GI microtissues grown in transwell plates that allow apical and/or basolateral drug treatment and 96-well throughput. Evaluation of assay utility focused on predictivity for diarrhea because this adverse effect correlates with intestinal barrier dysfunction which can be measured in GI microtissues using transepithelial electrical resistance (TEER). A validation set of widely prescribed drugs was assembled and tested for effects on TEER. When the resulting TEER inhibition potencies were adjusted for clinical exposure, a threshold was identified that distinguished drugs that induced clinical diarrhea from those that lack this liability. Microtissue TEER assay predictivity was further challenged with a smaller set of drugs whose clinical development was limited by diarrhea that was unexpected based on 1-month animal studies. Microtissue TEER accurately predicted diarrhea for each of these drugs. The label-free nature of TEER enabled repeated quantitation with sufficient precision to develop a mathematical model describing the temporal dynamics of barrier damage and recovery. This human 3D GI microtissue is the first *in vitro* assay with validated predictivity for diarrhea-inducing drugs. It should provide a platform for lead optimization and offers potential for dose schedule exploration.

Key words: gastrointestinal; toxicity; diarrhea; intestine; microtissue; enteroid; organoid.

Abstract: …Evaluation of assay utility focused on predictivity for diarrhea because this adverse effect correlates with intestinal barrier dysfunction which can be measured in GI microtissues using transepithelial electrical resistance (TEER). A validation set of widely prescribed drugs was assembled and tested for effects on TEER. When the resulting TEER inhibition potencies were adjusted for clinical exposure, a threshold was identified that distinguished drugs that induced clinical diarrhea from those that lack this liability. Microtissue TEER assay predictivity was further challenged with a smaller set of drugs whose clinical development was limited by diarrhea that was unexpected based on 1-month animal studies. Microtissue TEER accurately predicted diarrhea for each of these drugs. The label-free nature of TEER enabled repeated quantitation with sufficient precision to develop a mathematical model describing the temporal dynamics of barrier damage and recovery. This human 3D GI microtissue is the first *in vitro* assay with validated predictivity for diarrhea-inducing drugs. It should provide a platform for lead optimization and offers potential for dose schedule exploration.

Drug Safety: Gastrointestinal Toxicity (GI)

Diarrhea is common side effect in patients receiving oral small molecule tyrosine kinase inhibitors.

Treatment discontinuation and decreased drug efficacy.

Affect the pharmacokinetics of oral dosage regimens.

Rodent models: Incorrectly predict 50% of human GI toxicities.

Rodent and non-rodent models (combined**)** detects only 70% of human GI toxicities.

What is needed: Pre-clinical models which translate to clinical application.

EpiIntestinal
Gastrointestinal toxicity (GIT) Assessment – Animal Studies

Animal studies underpredict clinically observed diarrhea

Table 5. Nonmarketed Drugs With High Incidence of Clinical Diarrhea That Was Not Consistent Predicted by Preclinical 1-Month Animal Studies

CD	Clincical Diarrhea Incidence	Rat 1 Month Findings	Dog 1 Month Findings	EpiIntestinal Predicted GIT
AZD3409	41% (12/29)	GIT not noted at MTD	DLT: GIT	yes
AZD8931	51% (61/120)	DLT: GIT	GIT not noted at MTD	yes
AZD7140	60% (9/15)	GIT not noted at MTD	Occasion soft feces at highest dose	yes
AZD3	33% (8/24)	GIT not noted at MTD	GIT not noted at MTD	yes

Predictive clinical outcomes with human GI microtissue TEER were scored using TEER IC_{15}/C_{max} ratio < 80 as diarrhea-genic criteria. C_{max} valutes associated with diarrhea were used. Experiments were conducted under blinded conditions.

Test compounds: Non-marketed drugs with high Incidence of clinical diarrhea that were not predicted by preclinical 1-month animal studies.

Number of Compounds: N=4 drugs from the AstraZeneca collection that induced gastrointestinal toxicity (**GIT**).

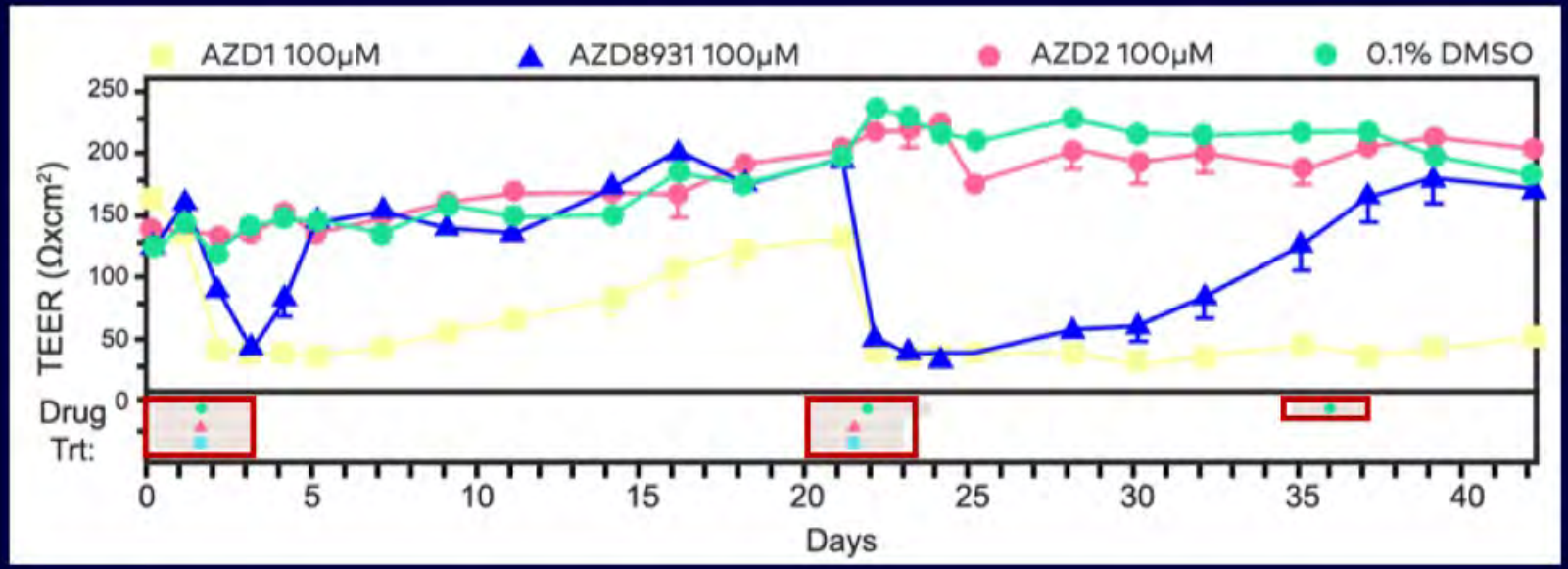
Criteria for selection: Availability of data from clinical exposure and preclinical animal tests (dose-limiting toxicity (**DLT**) or the most prevalent adverse effect (AE). Maximum Tolerated Dose (**MTD**).

Peters et al. Human ED Gastrointestinal Microtissue Barrier Function as a Predictor of Drug-Induced Diarrhea, ToxSci, 2018.

EpiIntestinal
GIT Assessment – Kinetic Studies

Key Data Points:

1) Inter-day consistency
2) Compatibility with drug washout
3) Viability for extended culturing
4) Compatibility with repeat dosing
5) Retain sensitivity to response on-set and recovery



Repeat dosing assessment

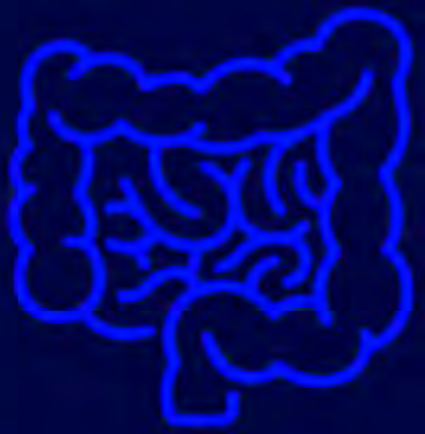
- AZD1 – proprietary candidate dose limited by diarrhea
- AZD2 – proprietary candidate not limited by diarrhea
- AZD8391 – positive control

Data demonstrating ability of EpiIntestinal to be used for kinetics and repeat dosing studies

Peters et al. Human ED Gastrointestinal Microtissue Barrier Function as a Predictor of Drug-Induced Diarrhea (ToxSci, 2018).

Tissue Characterization	• EpiIntestinal mimics the structure and function of human small intestine
	  ▪ Crypt-villus structures
	  ▪ Mucus producing goblet cells
	  ▪ LGR5+ stem cells for continued tissue regeneration
	• Lot-to-lot consistency

Culture Timeline	• The human primary cell-derived EpiIntestinal model can be cultured up to12 weeks • Repeat dosing and kinetic modeling possible
Exposure Options	• The tissue model is ideal for acute or chronic exposures • Apical and basolateral exposure possible
Drug Discovery	• Able to model both highly and poorly absorbed drugs similarly to in vivo • High metabolic enzyme expression like in vivo • Active transporters



Ready-to-use innovation driver

Basic research, proof of concept, development…

11,674 patents

with MATTEK technology used