Focus Group TRANSDERMAL AND MUCOSAL DELIVERY
CRS Controlled Release Society

Tough drug delivery, let's GUTting it out!

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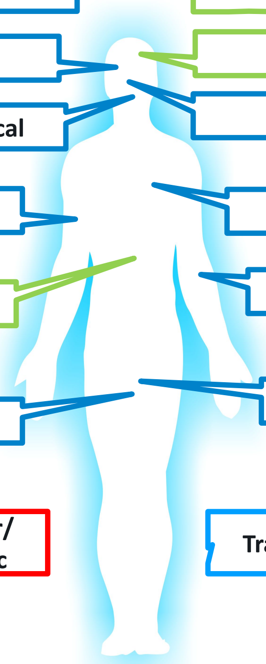
CRS 2022 Annual Meeting & Expo
July 11 – 15, 2022 | Montreal Congress Center, Montreal Canada

Advanced Delivery Science

Our motivation

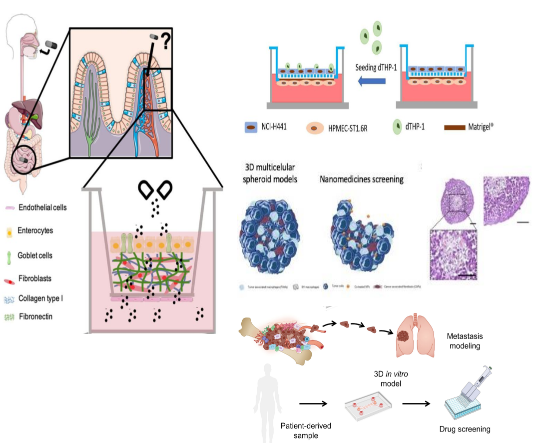


Delivery route

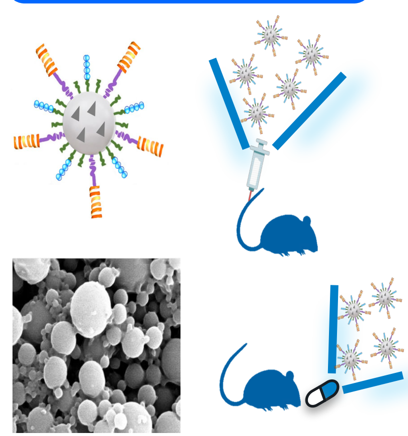
Drug target

- **Ocular** — *Int J Pharm, 572, 118811, 2019*
- **CNS** — *J Control Rel, 339, 381-390, 2021*
- **Oral/Buccal** — *Colloids Surf B Biointerfaces, 181, 149-157, 2019*
- **Nasal** — *J Control Rel, 309, 37-47, 2019*
- **Skin/Topical** — *Int J Pharm, 510, 221-231, 2016*
- **Pulmonary** — *Acta Biomat, 91, 235-247, 2019*
- **GIT** — *J Control Rel, 341, 430-342, 2022*; *J Control Rel, 327, 161-173, 2020*; *Eur J Pharm Sci, 137, 105011, 2019*
- **Intravenous** — *J Control Rel, 331, 198-212, 2021*; *Acta Biomat, 47, 71-80, 2017*
- **Vaginal** — *J Control Rel, 334, 453-462, 2021*; *Eur J Pharm Biopharm, 138, 3-10, 2019*
- **Rectal** — *ACS Applied Mat Interfs, 10, 34943-34952, 2018*; *Biomater Sci, 2019, 7, 38011-3811, 2019*
- **Intracellular/ Cell Specific** — *Bioact Mater, 9, 299-315, 2022*; *Biomaterials Science, 8, 3720-3729, 2020*; *Adv Funct Mat, 29, 1903686, 2019*
- **Translational** — *React Chem Eng, 5, 308-319, 2020*; *Nature Nanotechnology, 14, 629–635, 2019*; *Expert Opin Drug Discov, 14, 723-725, 2019*

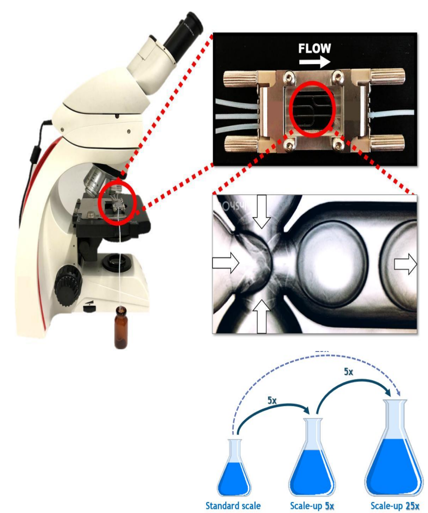
Biomimetic Barriers



Nanomedicines



Translational



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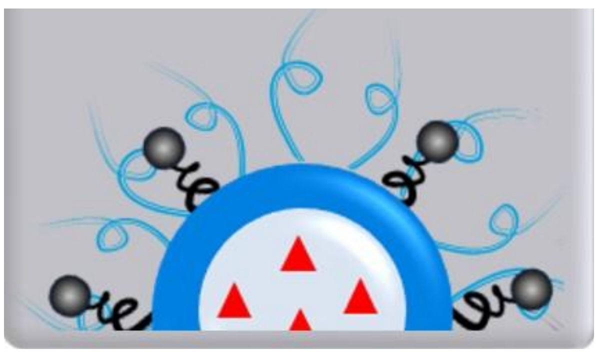
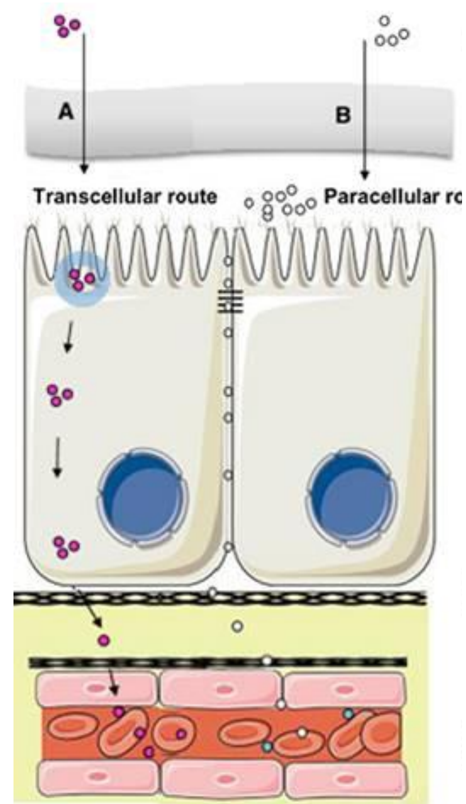
Our motivation – Mucosal Drug Delivery

Delivery Systems



Improvement of transport properties

Mucosal models



Reproduce drug permeability
Physiologically realistic architecture
Functional expression of transporters

Mucosal drug delivery

A promising and valid alternative to the parenteral route

- Non-invasive and painless administration
- Easy accessibility
- Rapid onset of action
- Elimination of the hepatic first-pass effect
- High bioavailability
- Cost effectiveness
- Flexibility in formulation design
- Self-administration
- Good patient compliance

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Oral drug delivery

A promising and valid alternative to the parenteral route

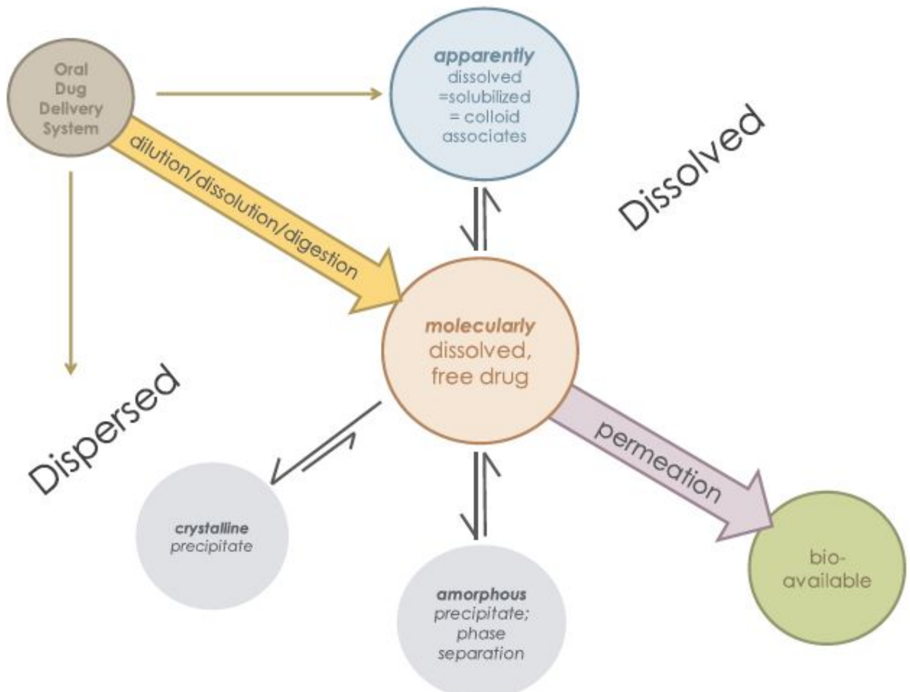
- Non-invasive and painless administration
- Easy accessibility
- Rapid onset of action
- Elimination of the hepatic first-pass effect
- High bioavailability
- Cost effectiveness
- Flexibility in formulation design
- Self-administration
- Good patient compliance

Oral drug delivery

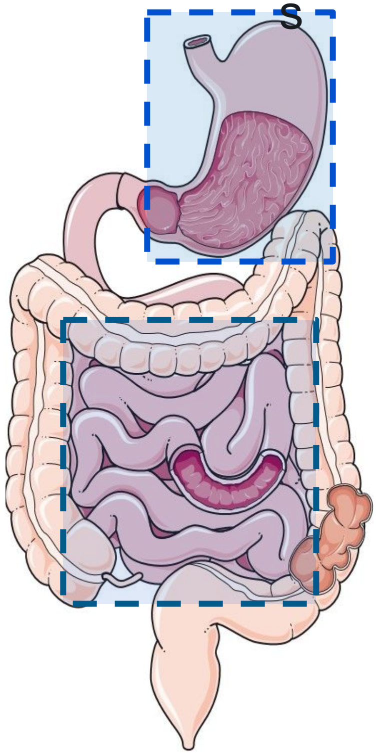
But…

Poor physical-chemical properties of drugs may lead to **low permeability**

Poorly water-soluble drugs



Biologics



Stomach

- Acidic pH
- Enzymatic degradation

Intestine

- Mucus layer
- Intestinal epithelia
- Enzymatic degradation

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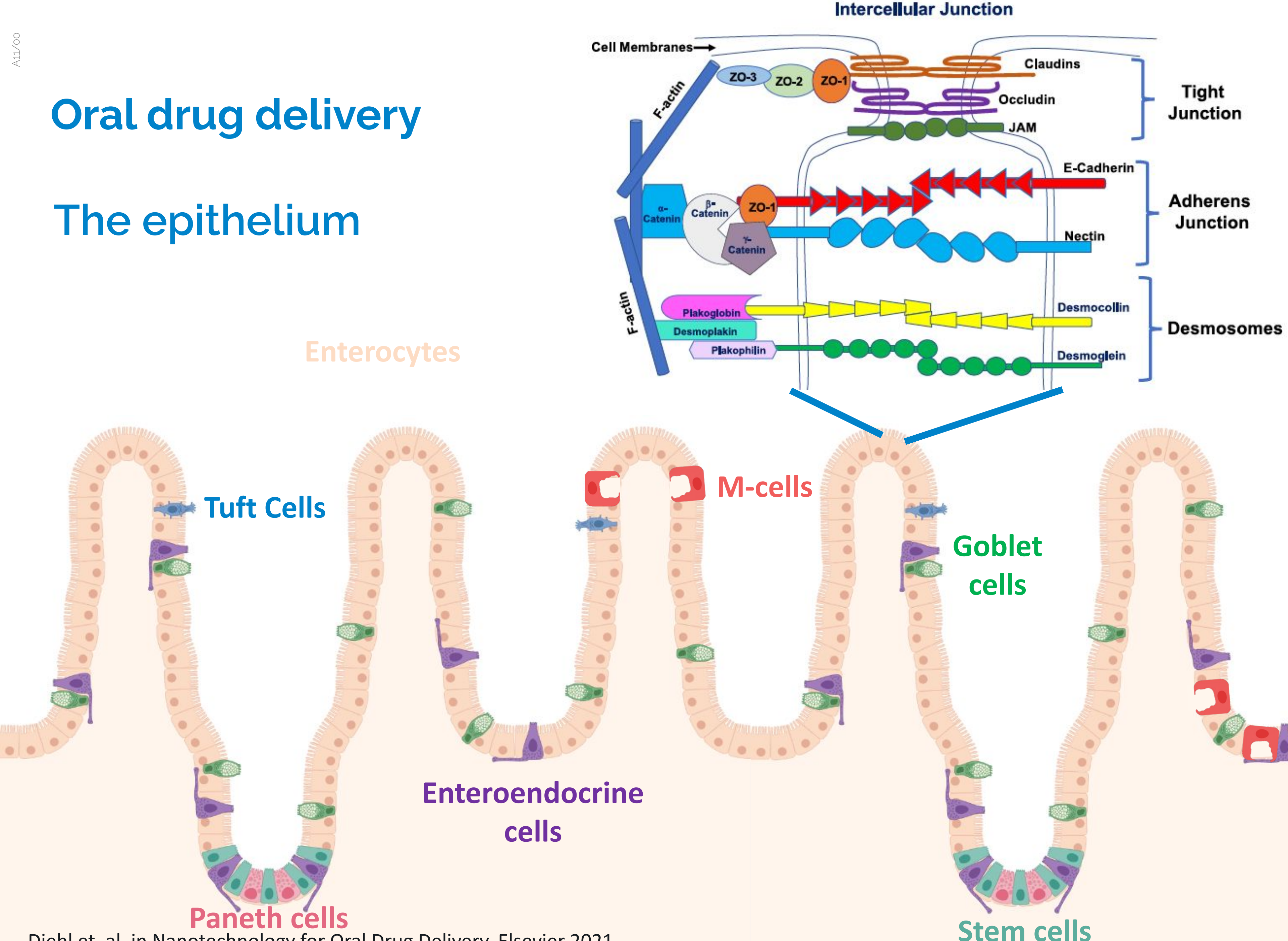
Oral drug delivery

The epithelium



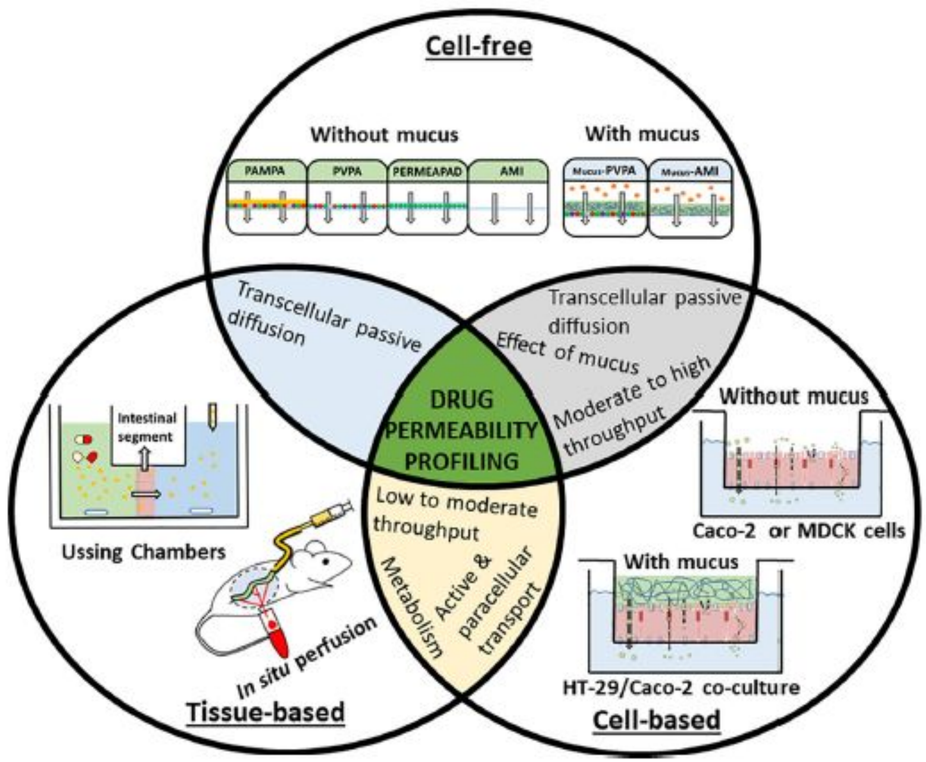
Diehl et. al, in Nanotechnology for Oral Drug Delivery, Elsevier 2021

Oral/Mucosal drug delivery

Formulation strategies

- Modification of molecular species
- Drug particle size reduction
- Amorphous dispersions
- Surfactants/emulsifiers
- Absorption enhancers
- Nanocrystals
- Nanodelivery systems

Validation models



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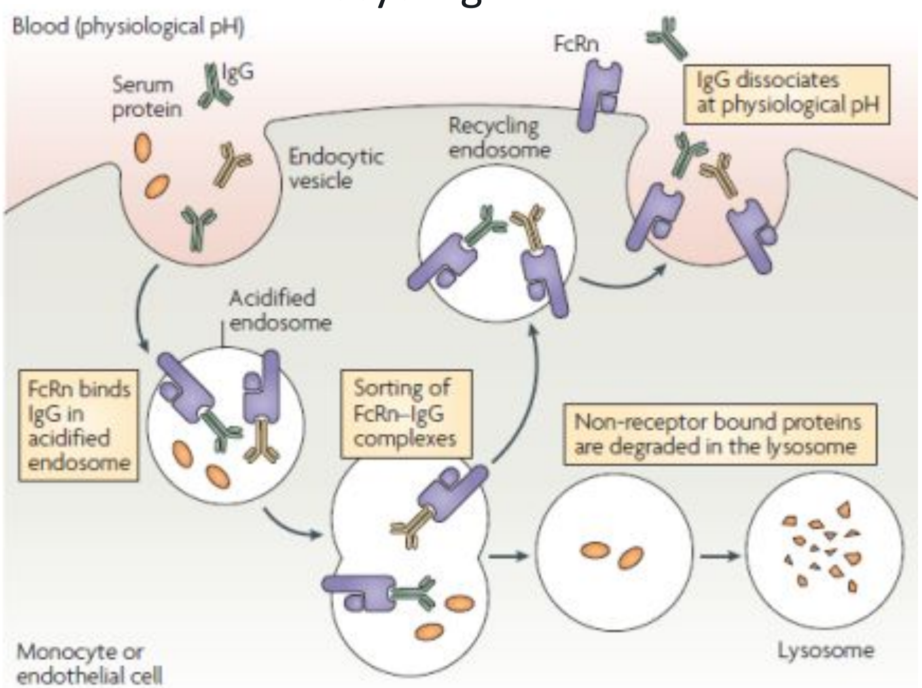
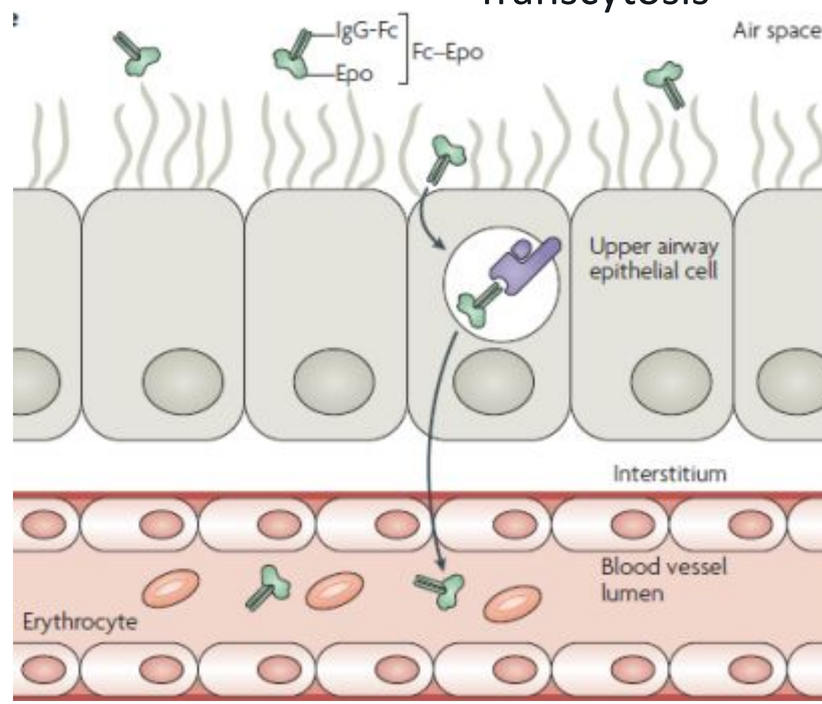
Functional nanoparticles for intestinal delivery through the FcRn transcytosis

- Transports IgG and HSA across cellular barriers
- Protects IgG and HSA from intracellular catabolism via strickly pH-dependent recycling and transcytosis pathway
- Homeostatic regulation, securing a broad biodistribution throughout the body

Recycling



Transcytosis



Roopenian D and Akilesh S, Nature Reviews 2007

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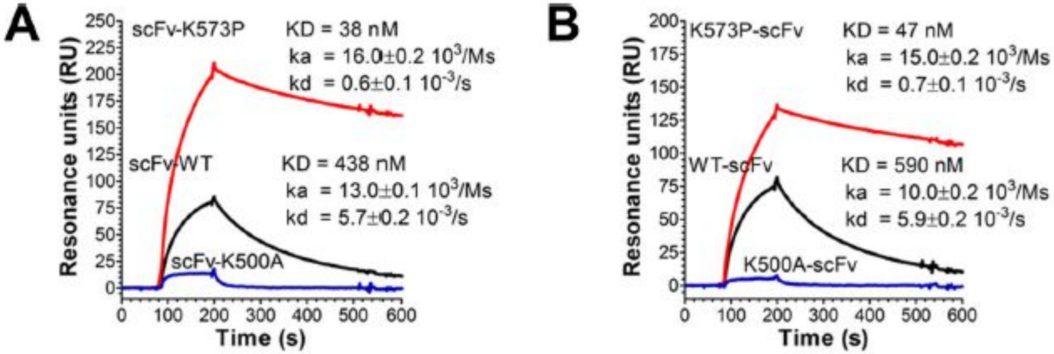
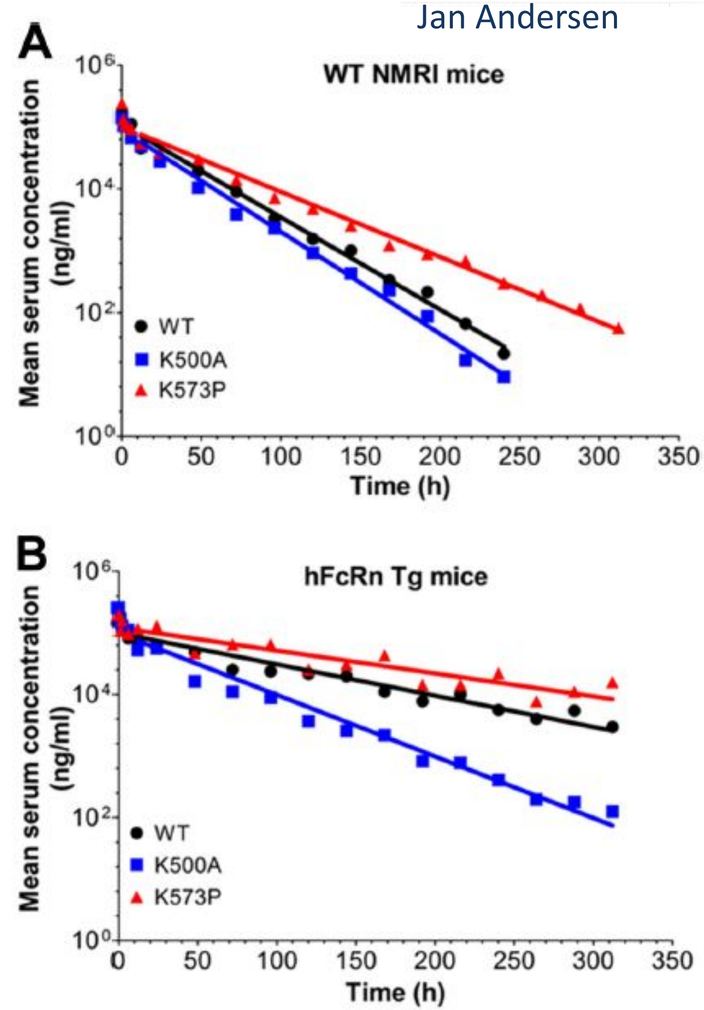
Functional nanoparticles for intestinal delivery of insulin through the FcRn transcytosis

Oslo University Hospital

Jan Andersen





HSA	Dose (i.v.)	C_{max}	AUC	V_z	CL	$T_{1/2}$
	mg/kg	*μg/ml*	*h × μg × ml*	*mg/kg*	*ml/h/kg*	*h*
WT NMRI mice						
WT	10	172	2,816	108.0	3.55	21.0
K500A	10	139	2,156	128.0	4.64	19.1
K573P	10	244	2,157	120.0	2.72	30.6
$FcRn^{-/-}$ hFcRn Tg32 mice						
WT	10	106	7,527	128.0	1.33	67.0
K500A	10	156	4,490	128.0	2.23	31.3
K573P	10	128	12,506	110.0	0.80	95.2

Andersen et. al., J Biological Chemistry 289, 13492–13502, 2014

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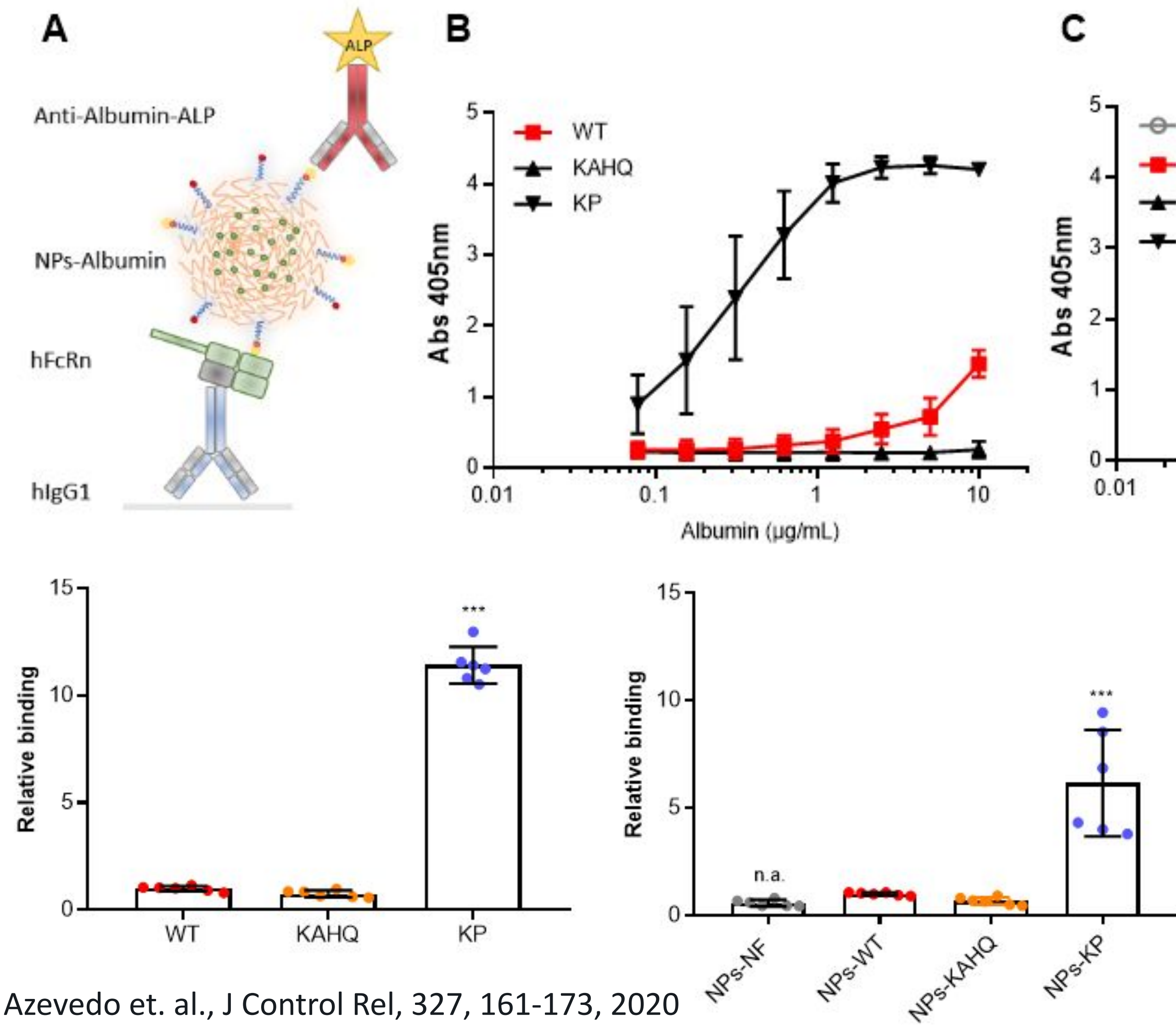
Functional nanoparticles for intestinal delivery of insulin through the FcRn transcytosis



NP
150 nm
-10 mV
10% Insulin loading

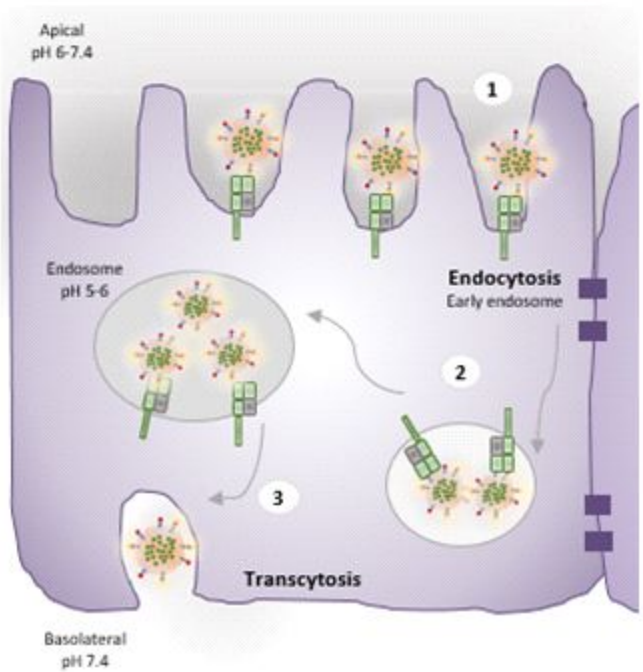
FcRn-binding properties of HSA were retained post conjugation

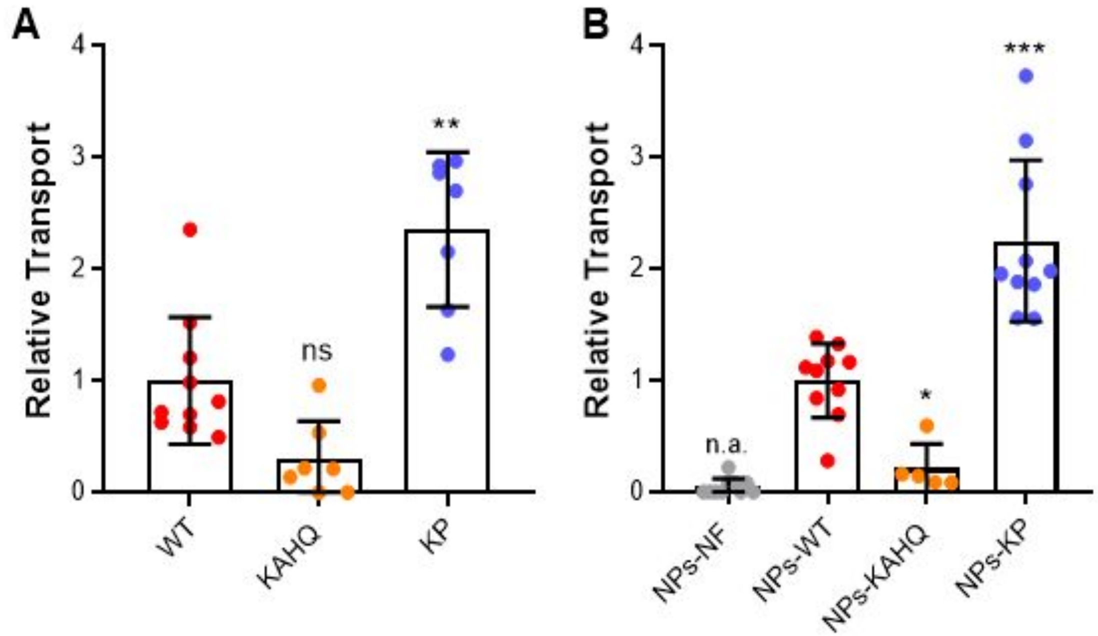
Azevedo et. al., J Control Rel, 327, 161-173, 2020

Functional nanoparticles for intestinal delivery of insulin through the FcRn transcytosis

MDCK-hFcRn cell line
pH 6

Transport





(1) The acidic pH at mucosal sites (apical) allows the NPs-HSA binding to FcRn at the cell surface;

(2) NPs-HSA are endocytosed to early endosomes;

(3) Endosomes fuse with the basolateral side and at neutral pH, FcRn releases the NPs-HSA.

Azevedo et. al., J Control Rel, 327, 161-173, 2020

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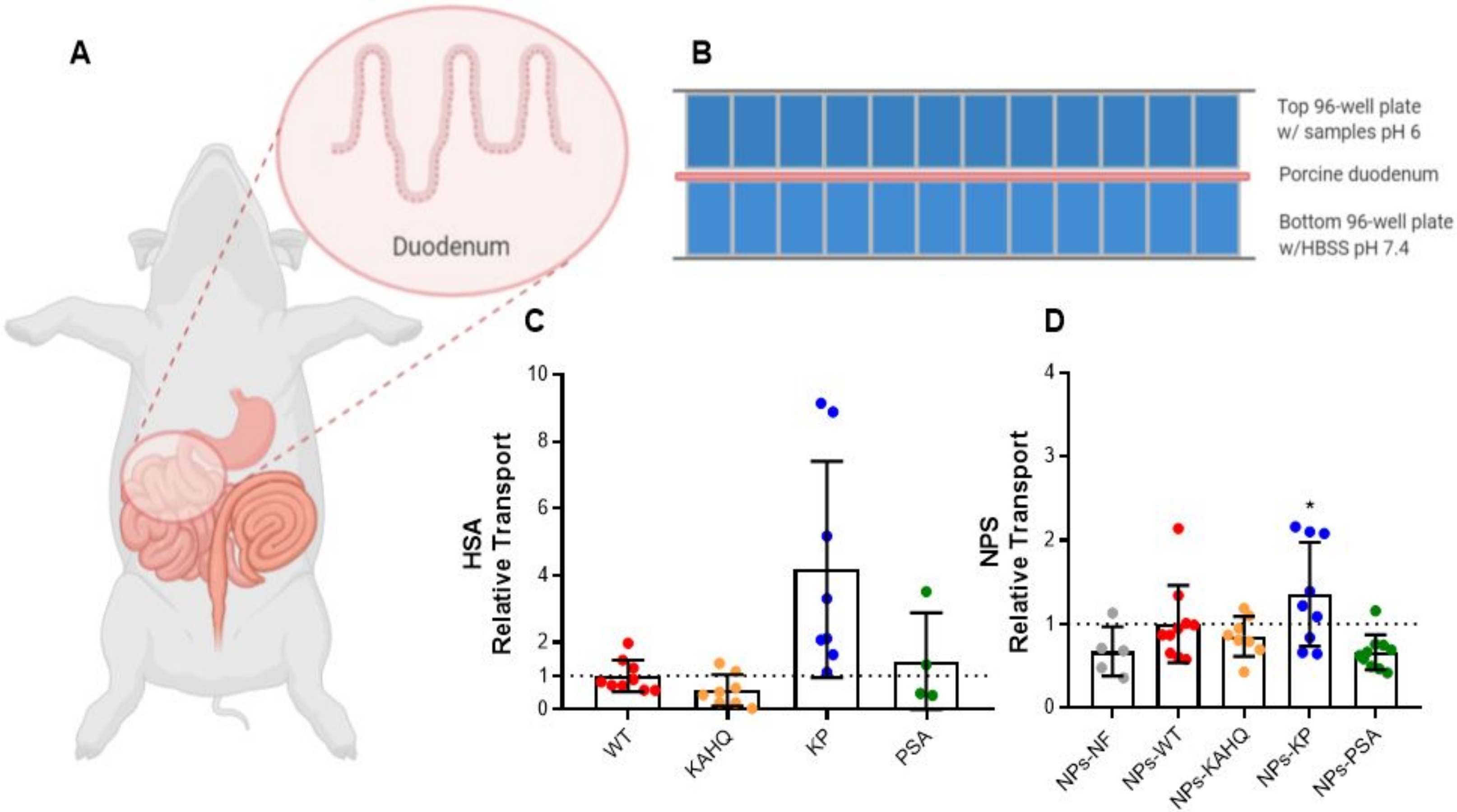
Functional nanoparticles for intestinal delivery of insulin through the FcRn transcytosis



Azevedo et. al., Eur J Pharm Biopharm, 162, 99-104, 2021

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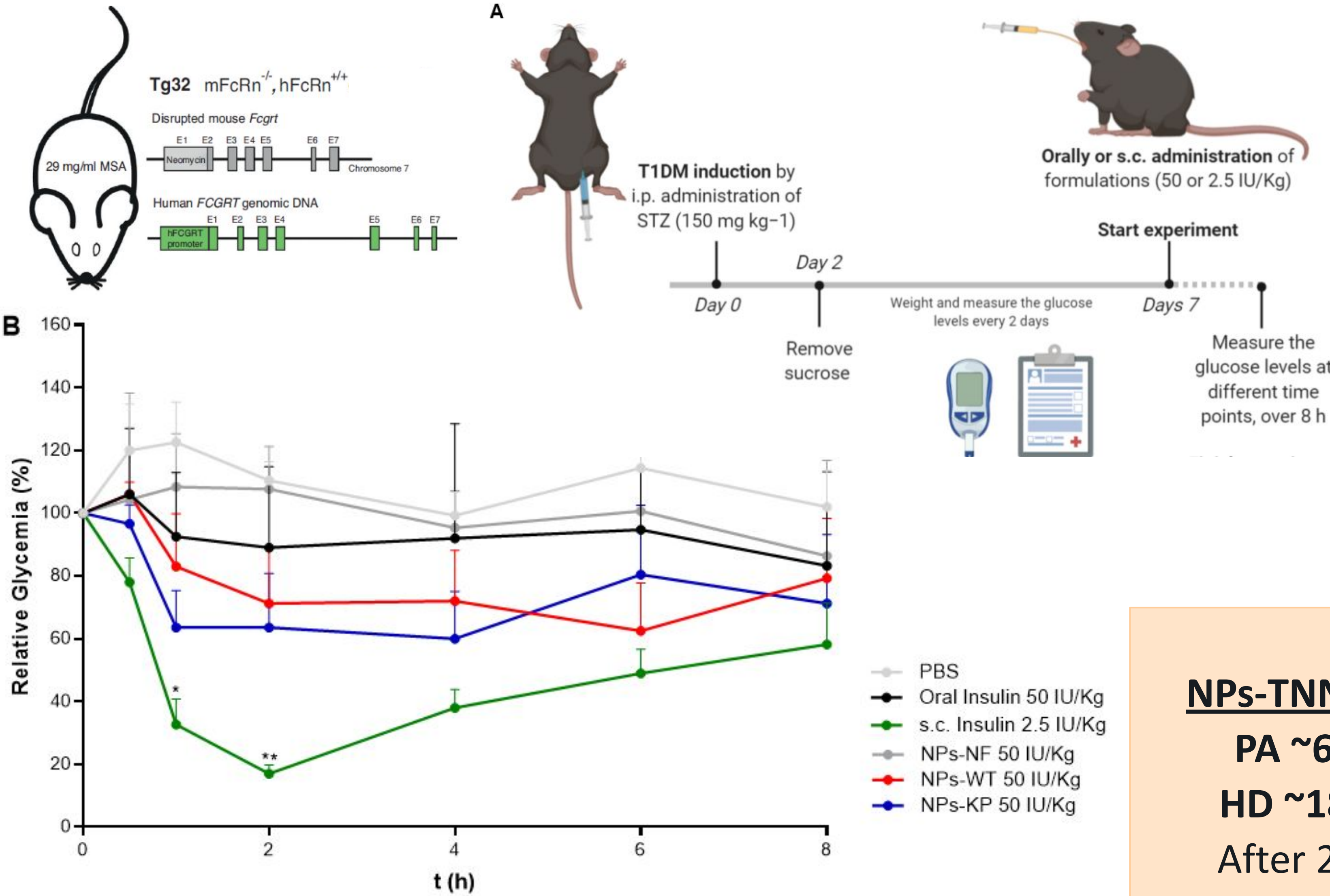
Functional nanoparticles for intestinal delivery of insulin through the FcRn transcytosis



Azevedo et. al., J Control Rel, 327, 161-173, 2020

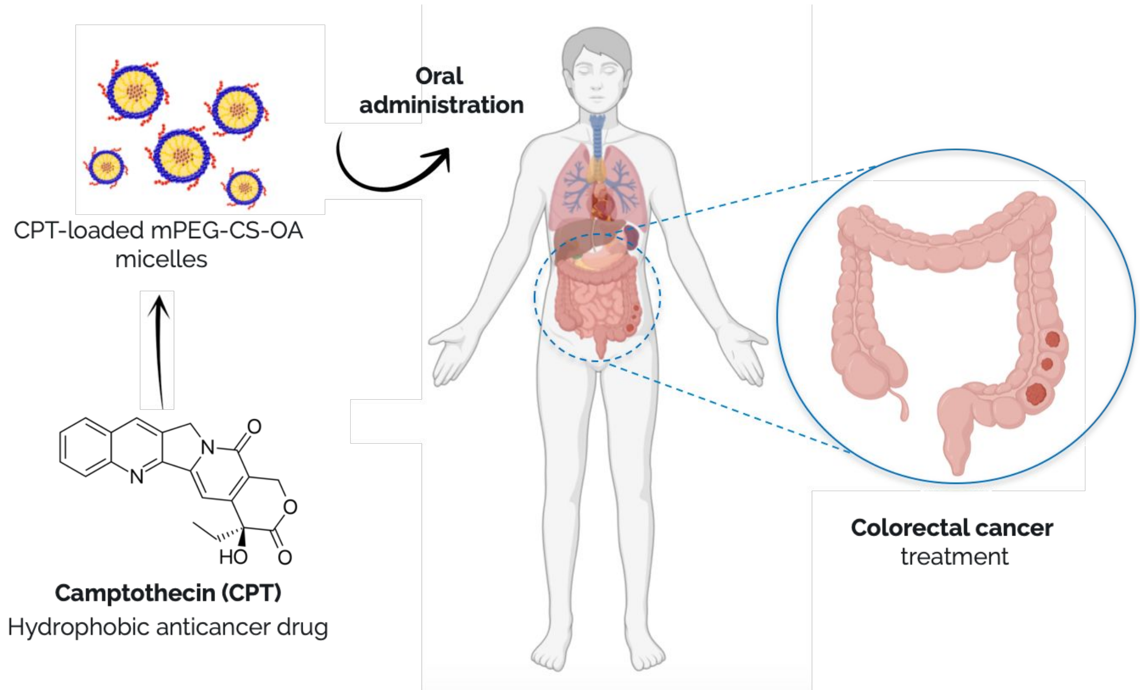
<u>NPs-TNNEKP</u>

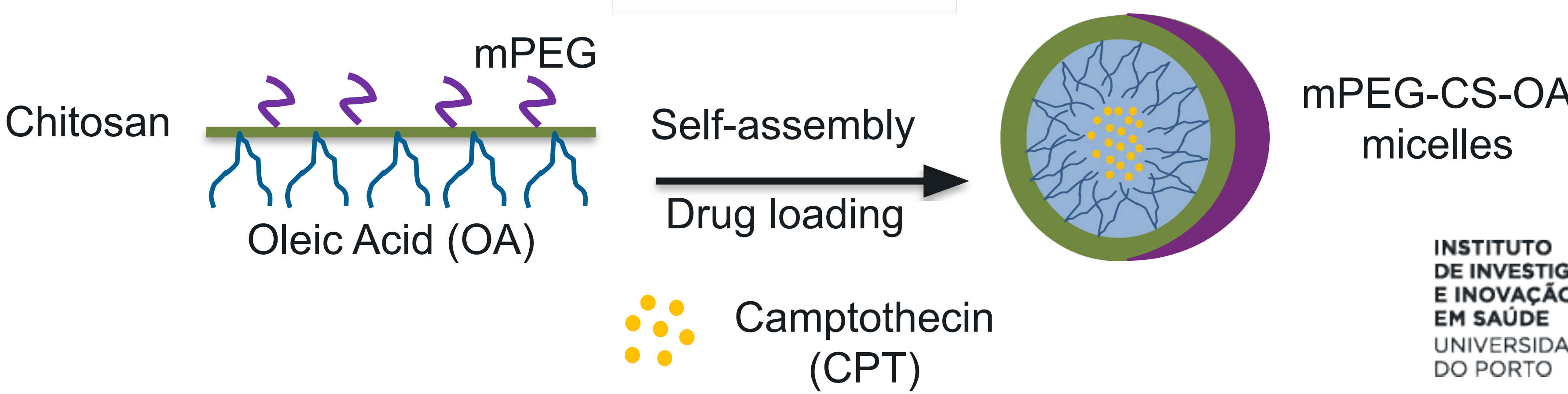
PA ~6%

HD ~18%

After 24h

Polymeric micelles for oral chemotherapy to treat colorectal cancer





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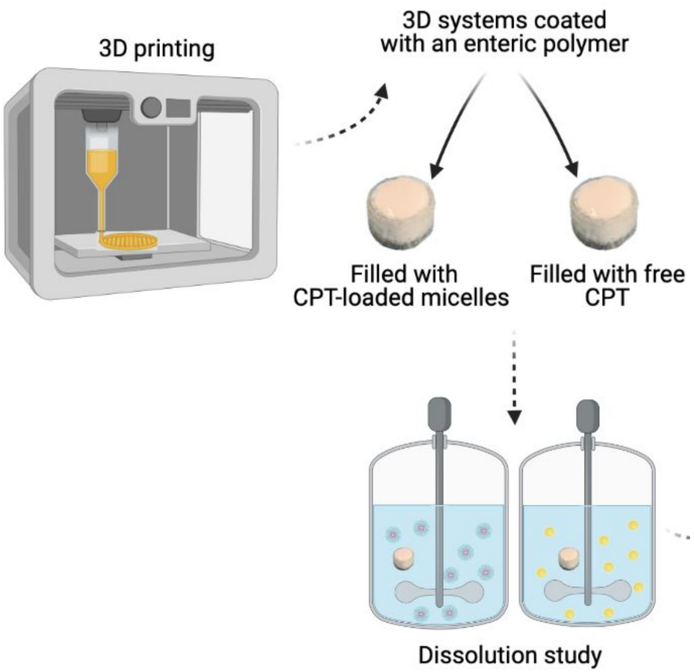
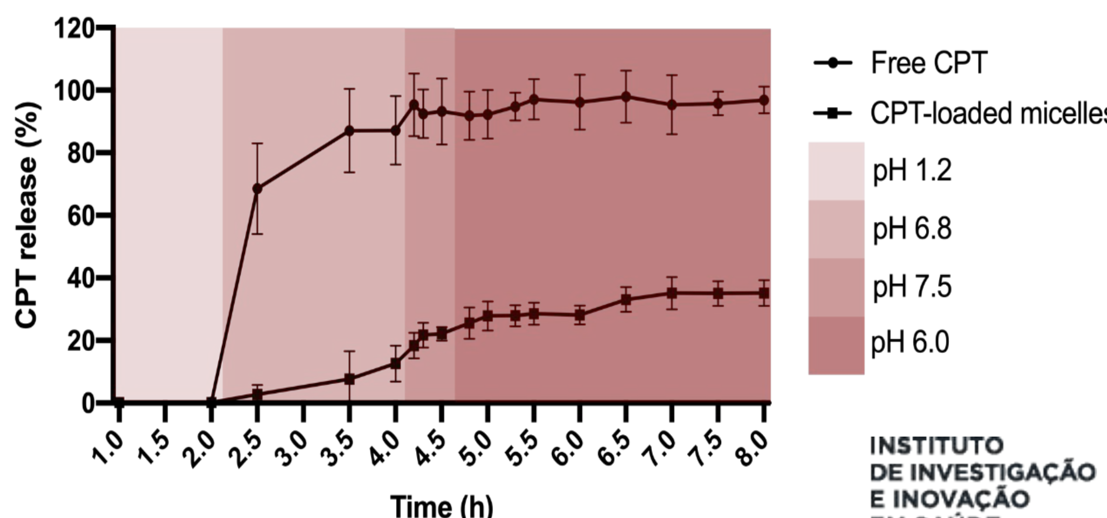
Polymeric micelles for oral chemotherapy to treat colorectal cancer

	Size (nm)	PdI	Zeta potential (mV)	DL (%)	AE (%)
Unloaded mPEG-CS-OA	137 ± 5	0.233 ± 0.025	+ 33.7 ± 1.8	0.0	-
CPT-loaded mPEG-CS-OA	146 ± 3	0.229 ± 0.005	+ 41.8 ± 3.0	5.0	78 ± 8





Almeida, A., et al, Eur J Pharm Biopharm, 167, 48-56, 2021
Almeida, A., et al., Materials and Science Engineering C, 2020

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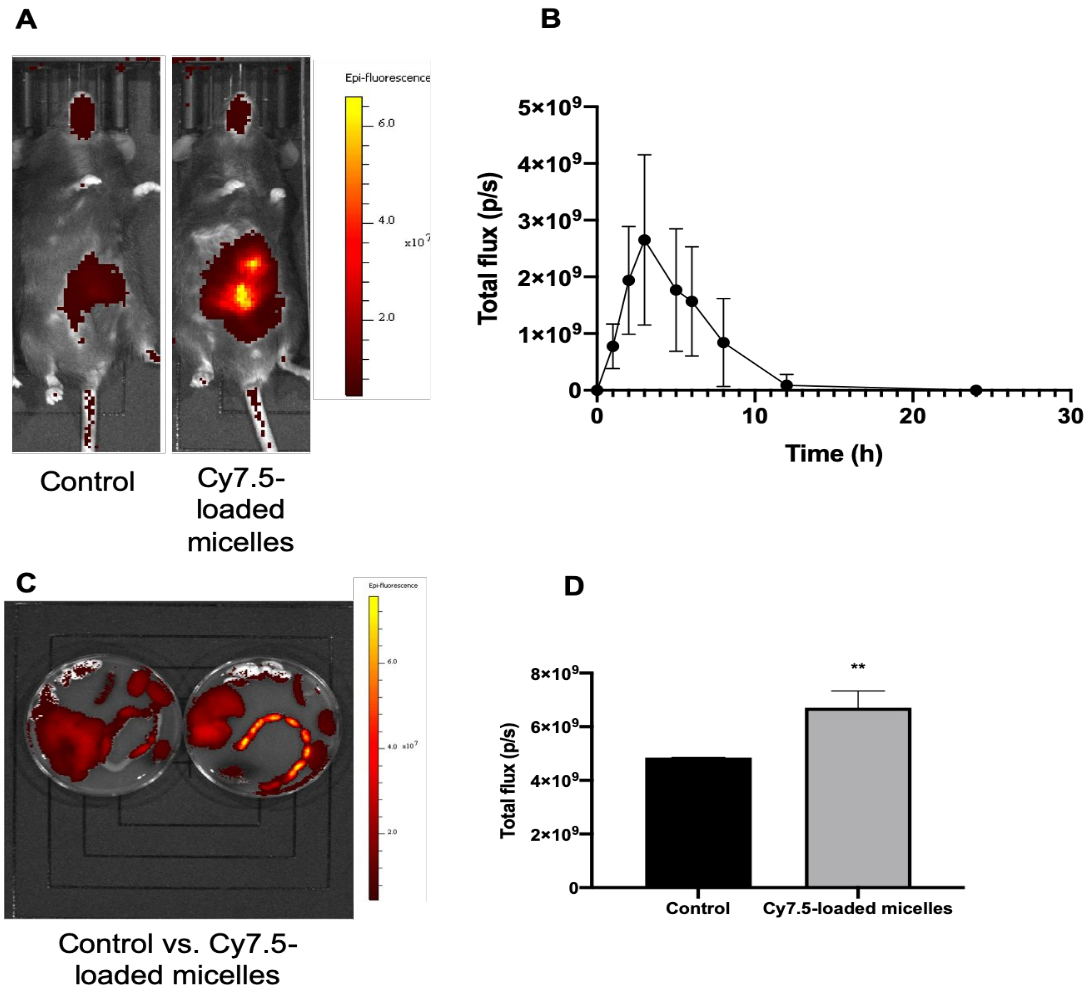
AOM/DSS CRC mouse model

Polymeric micelles *in vivo* biodistribution



Assessment of the distribution and retention of fluorescent chitosan micelles following oral administration by NIR imaging.

Almeida, A., et al., J Control Rel, accepted

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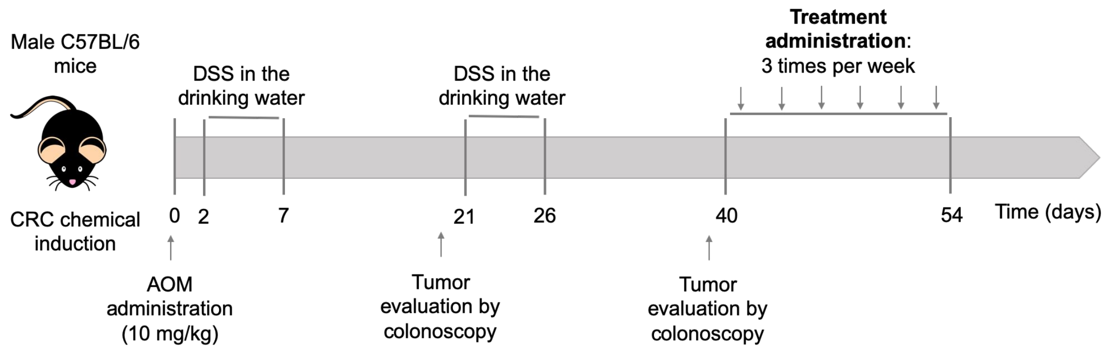
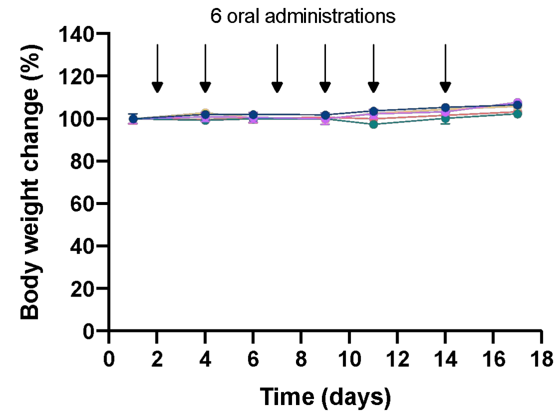
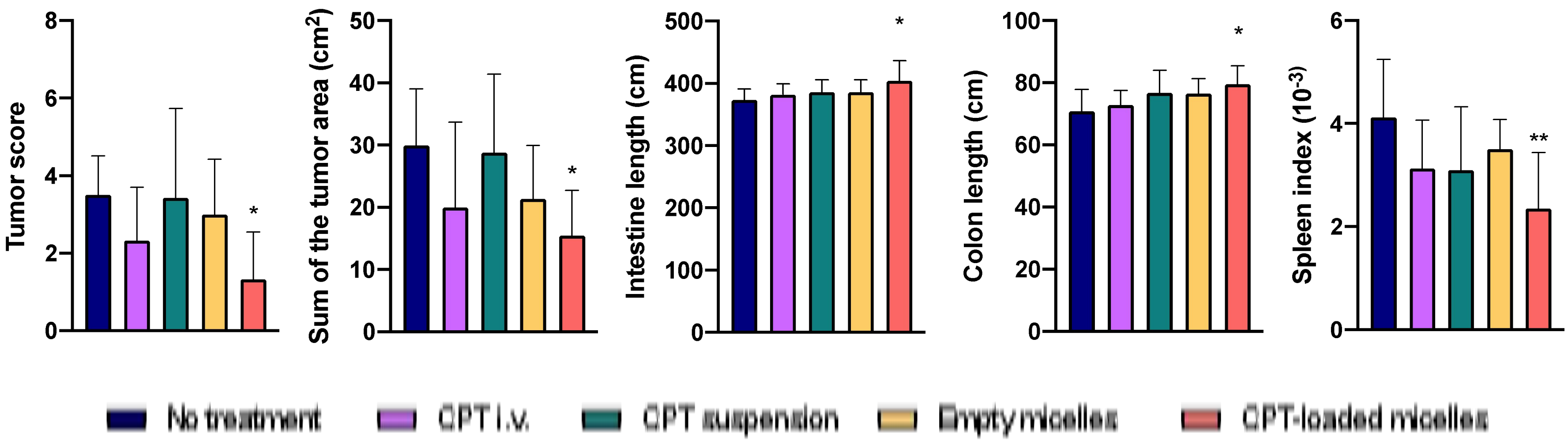
AOM/DSS CRC mouse model

Polymeric micelles *in vivo* anticancer activity





CPT suspension and CPT-loaded micelles = 1.5 mg/Kg
CPT i.v. = 0.1 mg/Kg



Almeida, A., et al., J Control Rel, accepted

3D cell-based *in vitro* models in drug delivery

- Rapid, cost-effective, and adequate predictability of drug PK/PD
- Less amount of drug is needed for the assay
- More compounds can be screened
- Mechanism of transport and metabolism can be studied
- Gene and protein expression
- Microscale tissue architecture/Biochemical gradients
- The analytical evaluation is simpler compared to assays in biological fluids
- Offers reproducibility and simplicity
- Few or no animals are used (in line with the "three Rs" ethical)

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The endothelial membrane in permeability

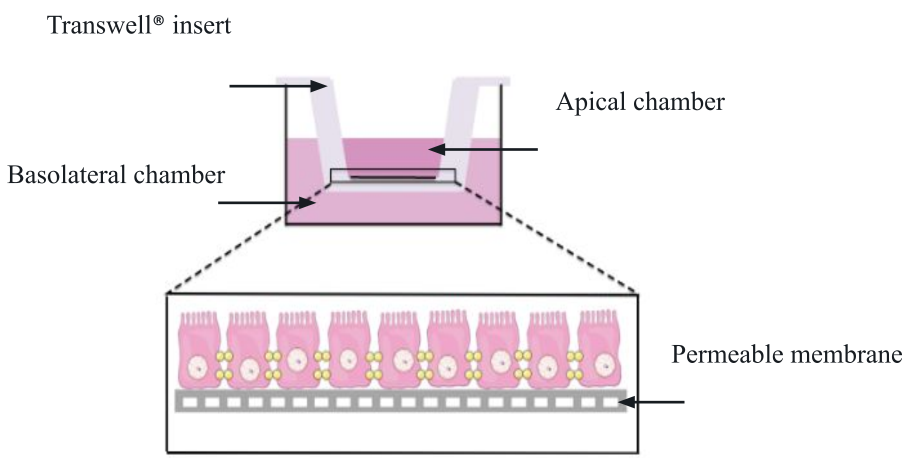
Vascular permeability in mucosa tissues

- Microvessels capillaries associated with absorptive mucosa (intestine) are fenestrated endothelia (40-70 nm) for nutrient absorption purposes
- Single continuous layer of endothelial cells joined by tight junctions and surrounded by a continuous basement membrane
- Endothelial cells control the passage of antigens and commensal gut microbiota from the intestine into the bloodstream (Science 350, 830 (2015))

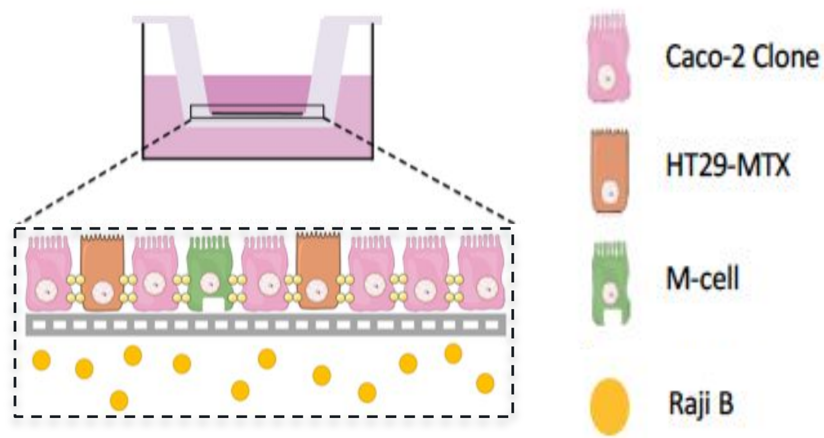
Intestinal models to perform permeability studies

	In Vivo		In Vitro
Absorptive lineage	Enterocytes	←	Caco-2 cells
Secretory lineage	Goblet cells	←	HT29-MTX cells
	Enteroendocrine cells		
	Paneth cells		
	M cells	←	Raji B lymphocytes
	Tuft cells		
	Cup cells		

“gold-standard” Caco- 2 model



Triple model



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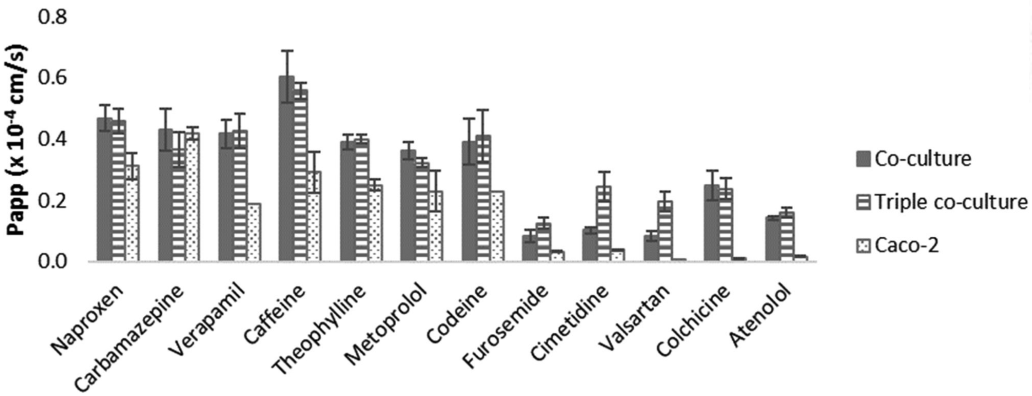
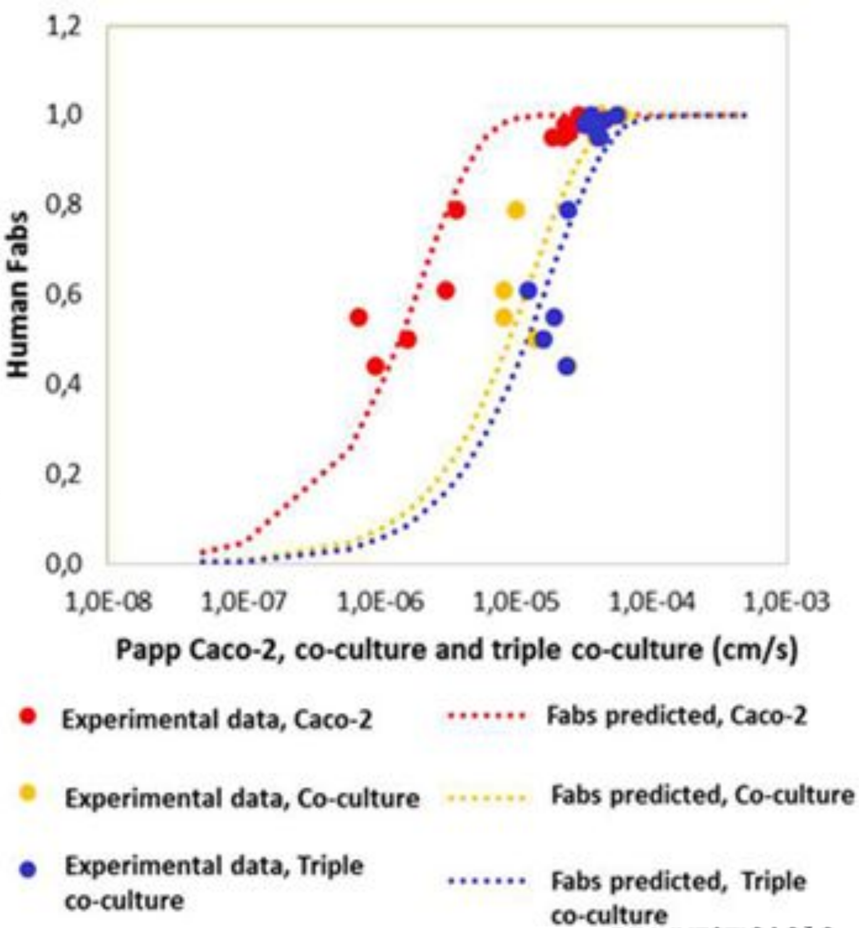
Antunes et al, Eur J Pharm Biopharm, 83, 427-435, 2013
Almeida et. al, in Nanotechnology for Oral Drug Delivery, 2020

In vitro intestinal co-culture epithelium model
In vitro/In vivo correlation





Lozoya-Agullo et. al, Mol. Pharmaceutics, 14, 1264−1270, 2017

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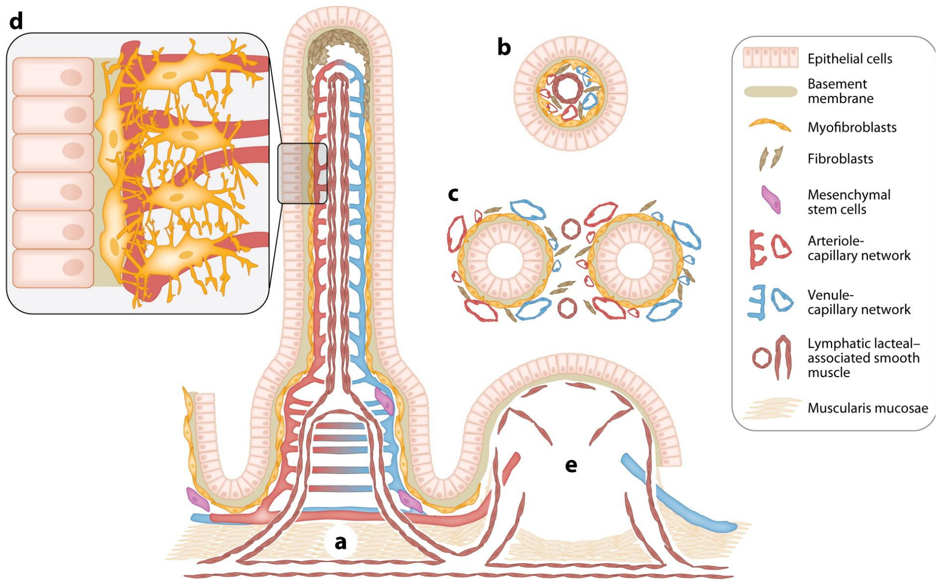
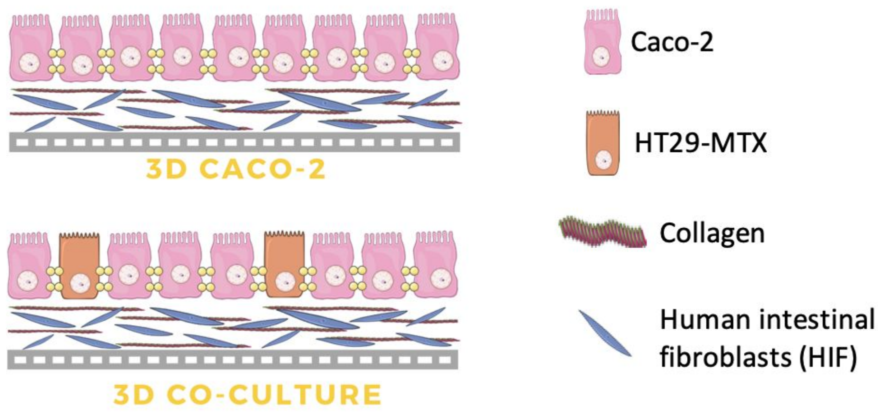
In vitro intestinal 3D epithelium model

The lamina propria



Powell DW, et al. 2011.
Annu. Rev. Physiol. 73:213–37



Collagen concentration – 6 mg/mL

HIF initial seeding density – 1 x 10^5 cells/mL

Caco-2 - 1 x 10^5 cells/cm^2

Co-culture - 1 x 10^5 cells/cm^2 (9:1 Caco-2:HT29-MTX)

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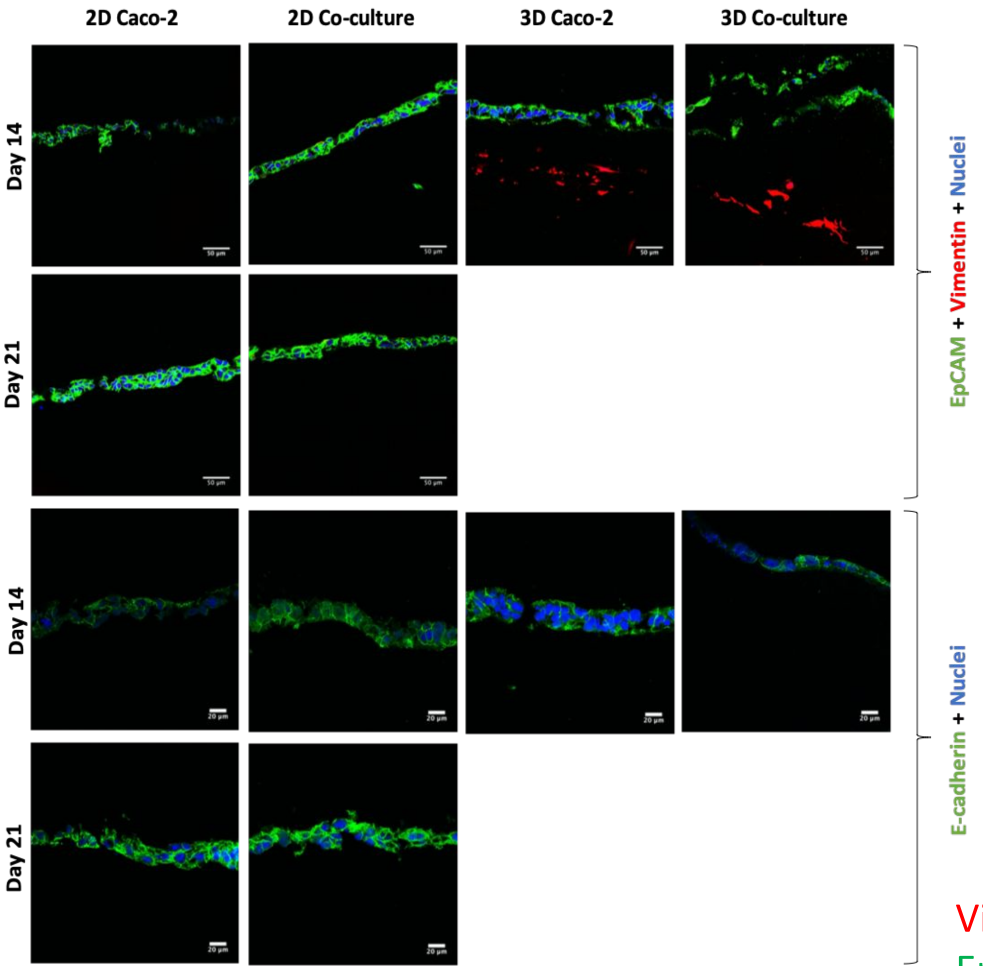
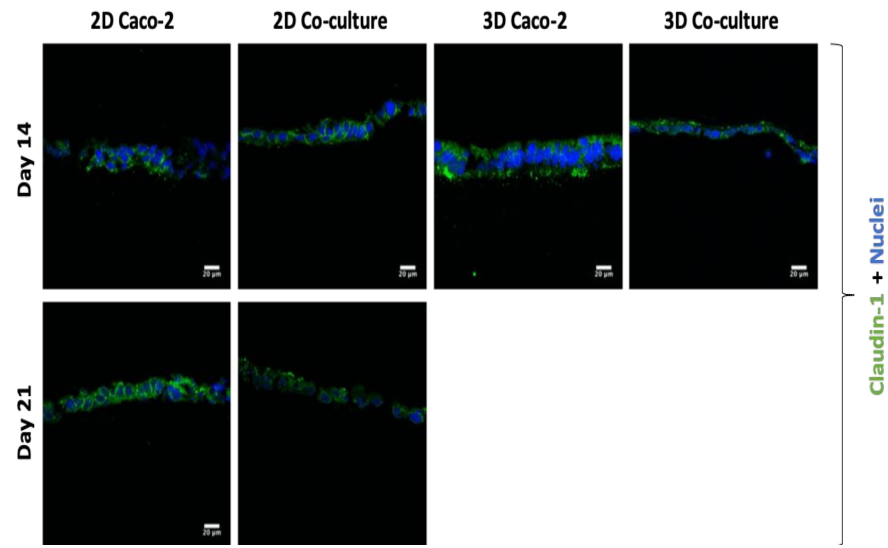
Macedo, MH. et al, Front Bioeng Biotechnol, 524018, 2020

In vitro intestinal 3D epithelium model

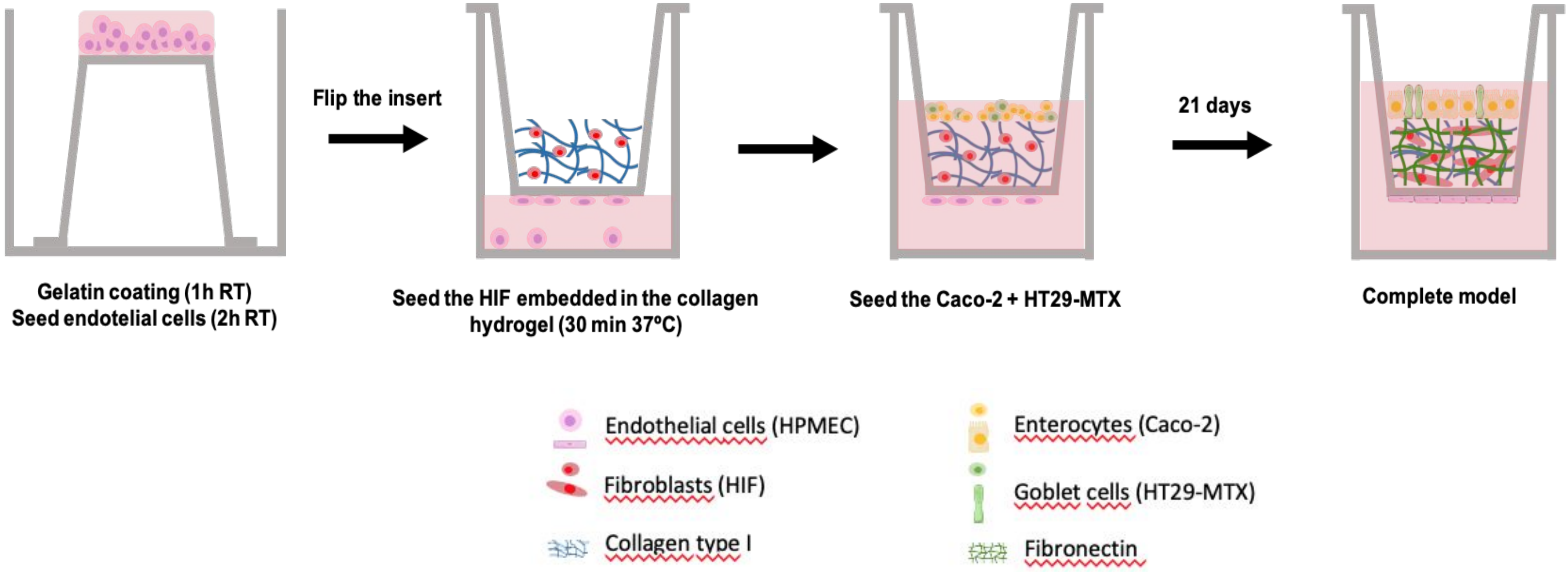




Vimentin - cytoskeletal intermediate filament
EpCAM - epithelial cell adhesion glycoprotein
E-cadherin - glycoprotein involved in cell-cell adhesion
Claudin-3 - transmembrane proteins of tight junctions

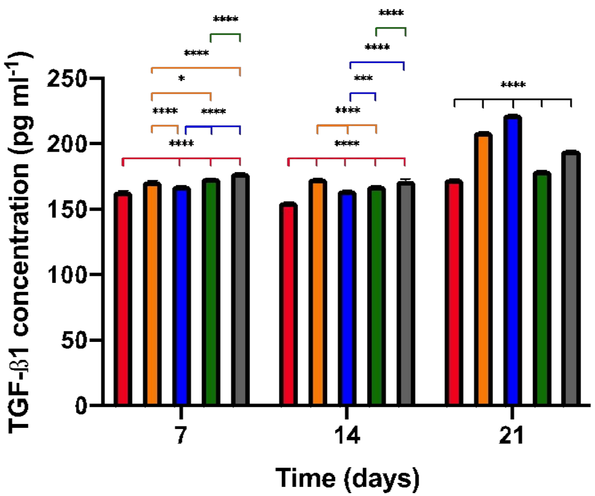
Macedo, MH. et al, Front Bioeng Biotechnol, 524018, 2020

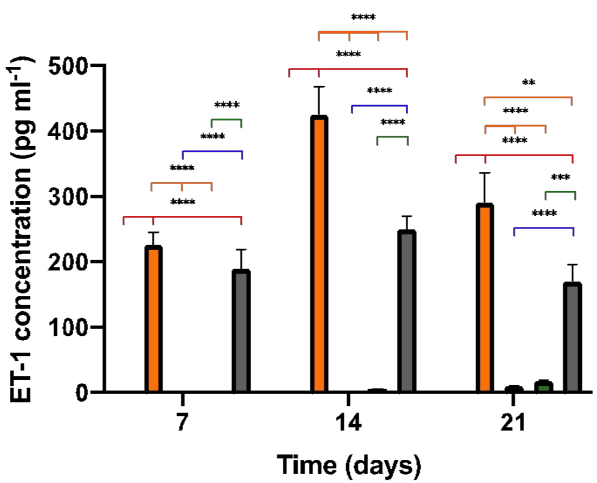
In vitro intestinal 3D epithelium model

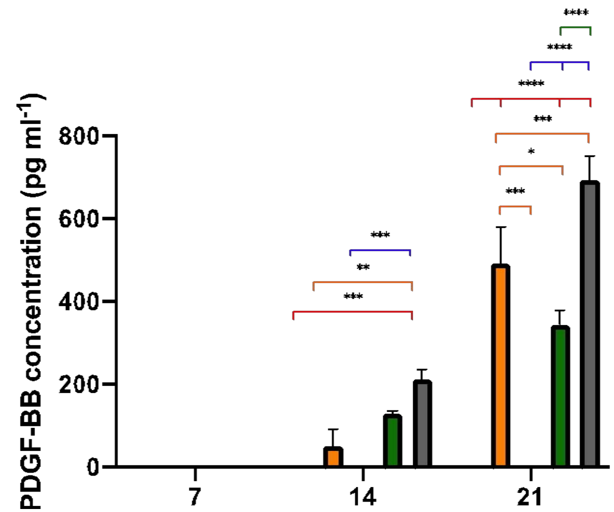


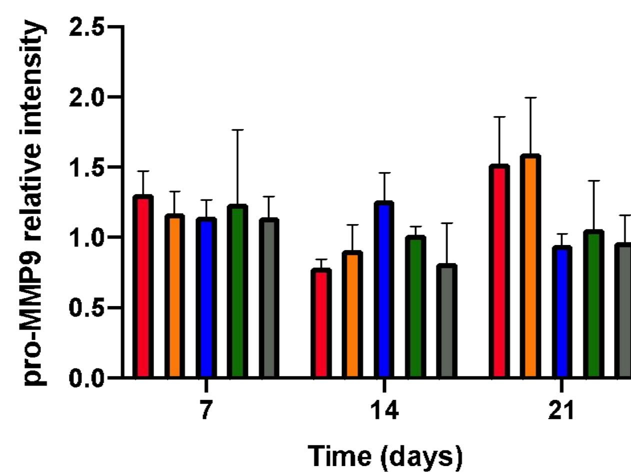
In vitro intestinal 3D epithelium model

How do the other cells in the model influence the contractibility of fibroblasts?









- HIF
- HIF + HPMEC
- HIF + Caco-2 clone
- HIF + Caco-2 + HT29-MTX
- HIF + Caco-2 + HT29-MTX + HPMEC

TGF - Transforming Growth Factor

ET – Endothelin

PDGF – Platelet Derived Growth Factor

Macedo. et al, J Control Rel 341, 430-342, 2022

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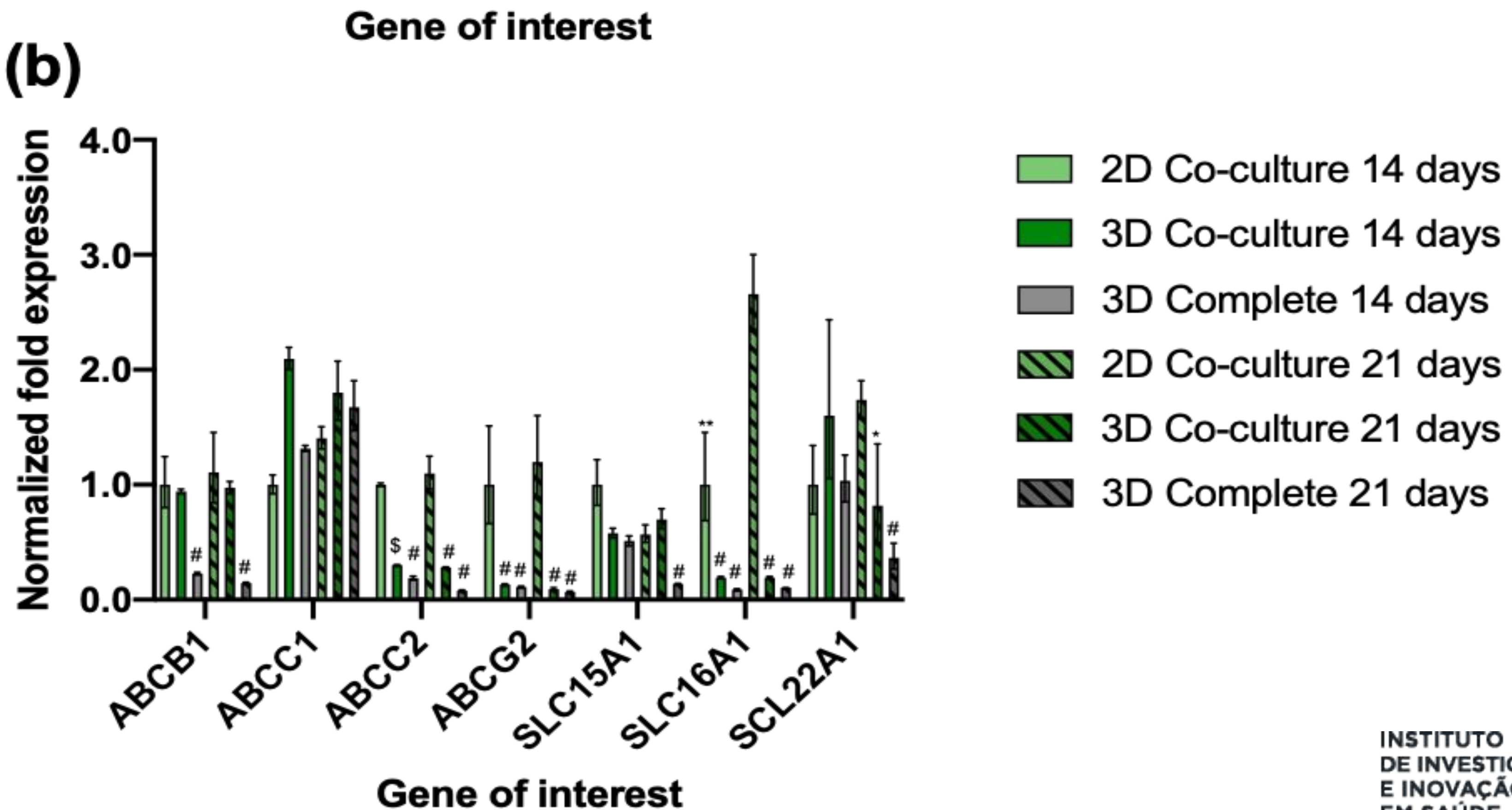
In vitro intestinal 3D epithelium model



Macedo. et al, J Control Rel 341, 430-342, 2022

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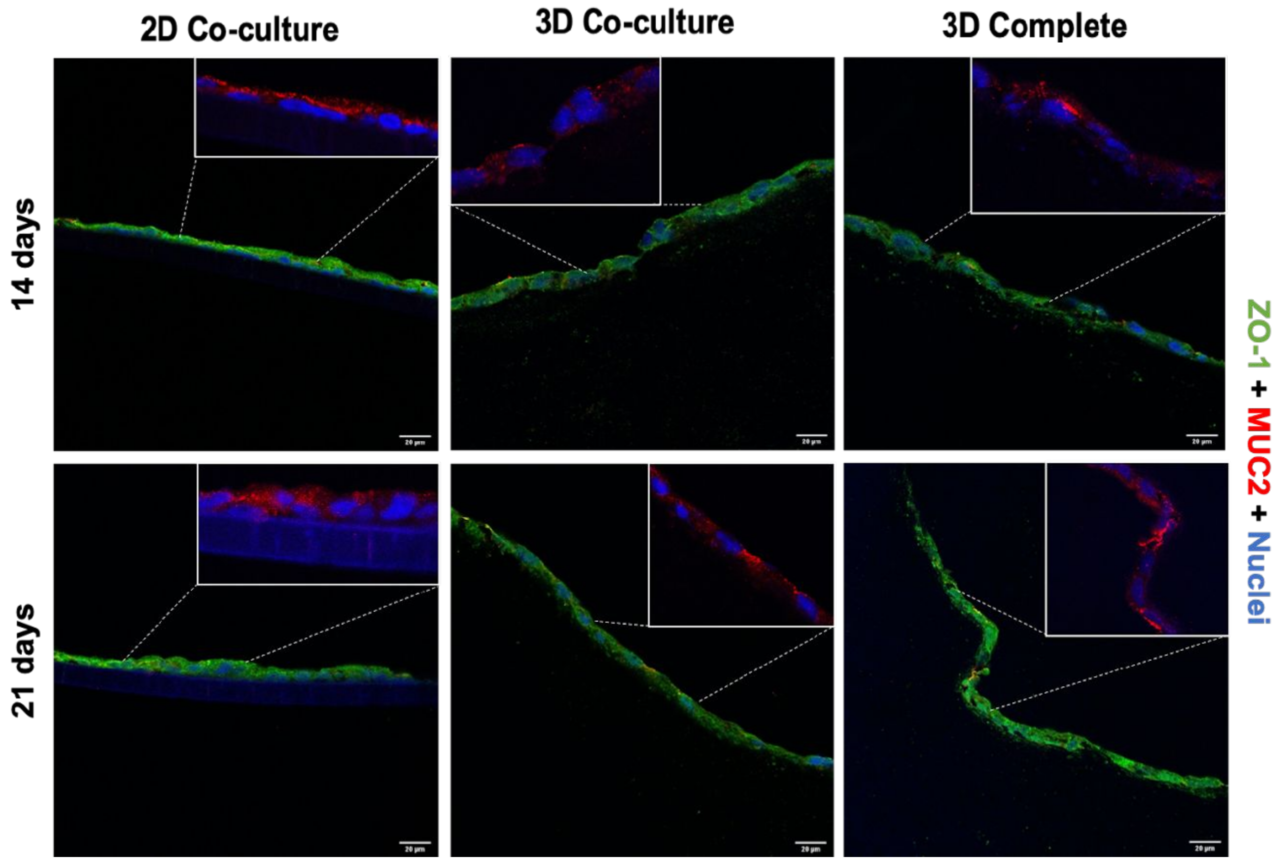
In vitro intestinal 3D epithelium model

Formation of endothelial barrier - Expression of TJs and MUC2



Macedo. et al, J Control Rel 341, 430-342, 2022

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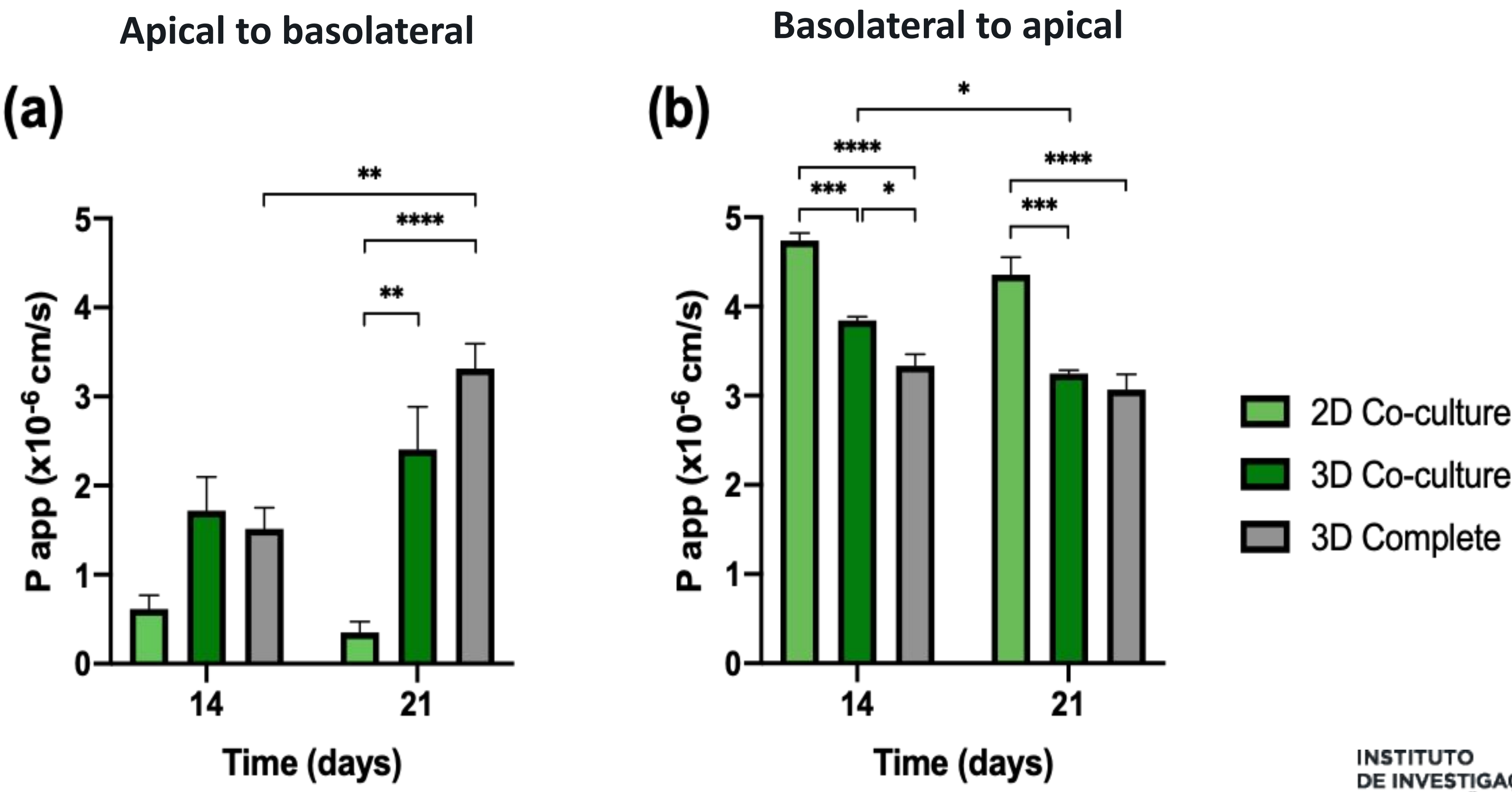
In vitro intestinal 3D epithelium model

Permeability of Rhodamine 123 – P-gp activity



Macedo. et al, J Control Rel 341, 430-342, 2022

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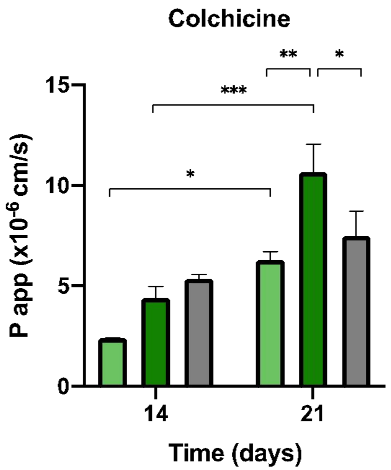
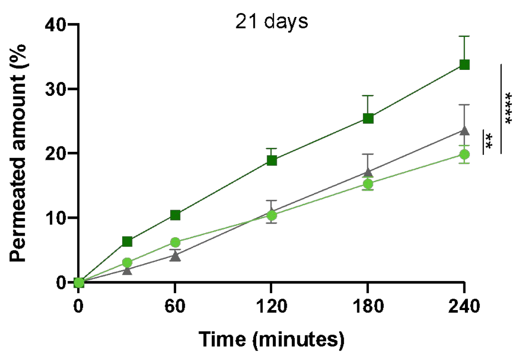
In vitro intestinal 3D epithelium model

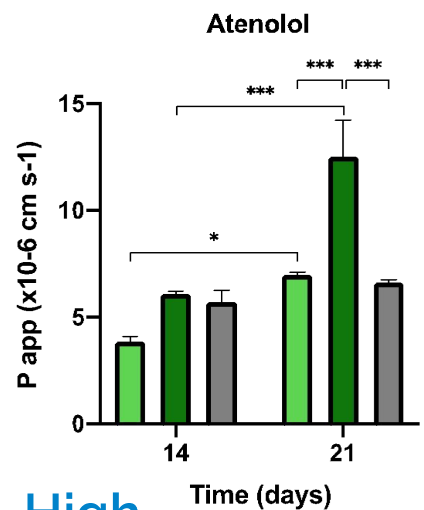
Permeability

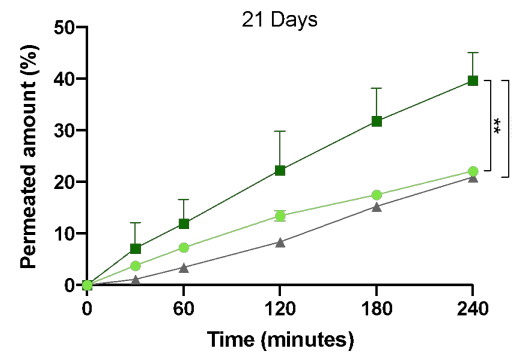
Low



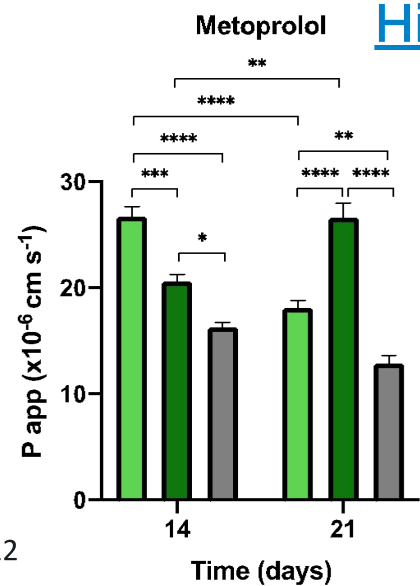


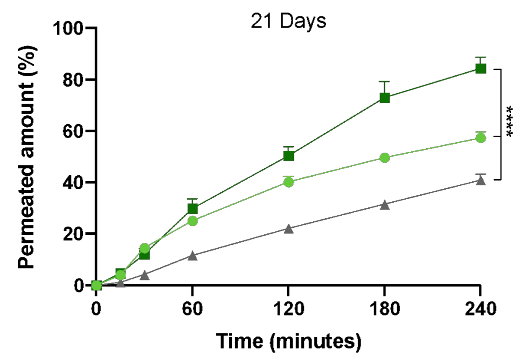
Moderate





High





2D Co-culture
3D Co-culture
3D Complete

Macedo. et al, J Control Rel 341, 430-342, 2022

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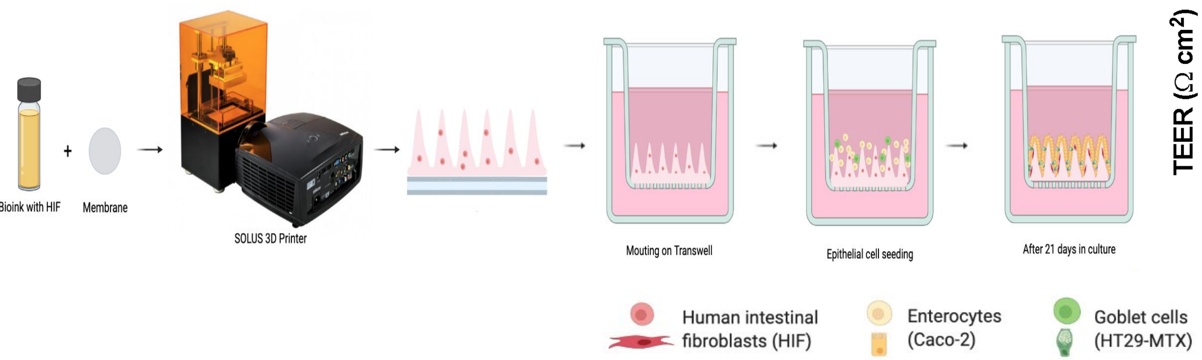
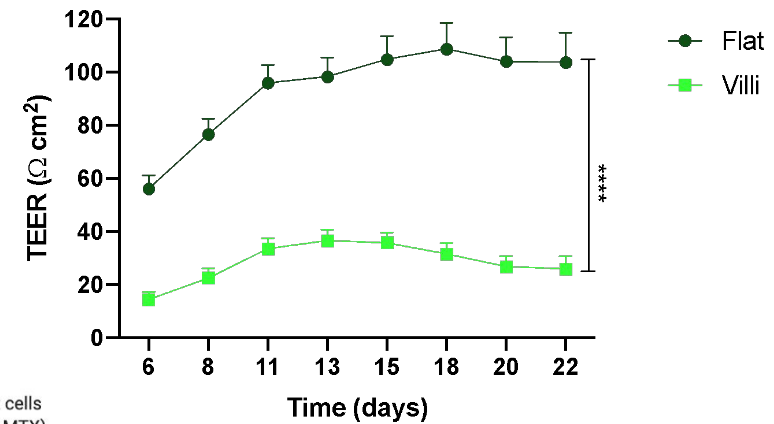
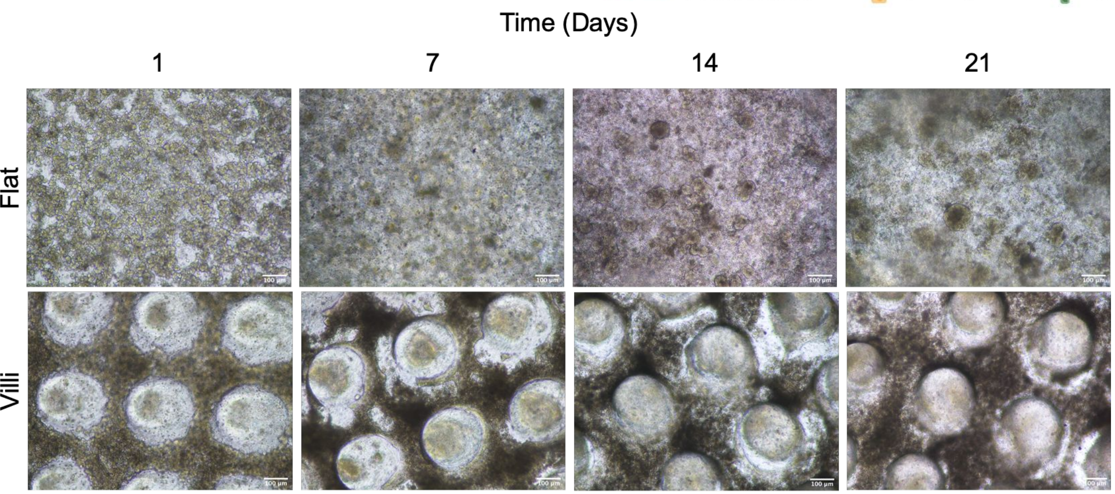
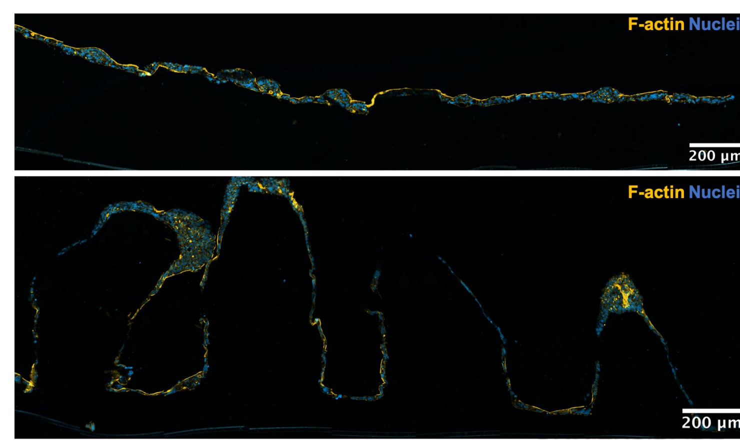
In vitro intestinal 3D epithelium model

The importance of the villi architecture in a 3D bioprinted in vitro intestinal model







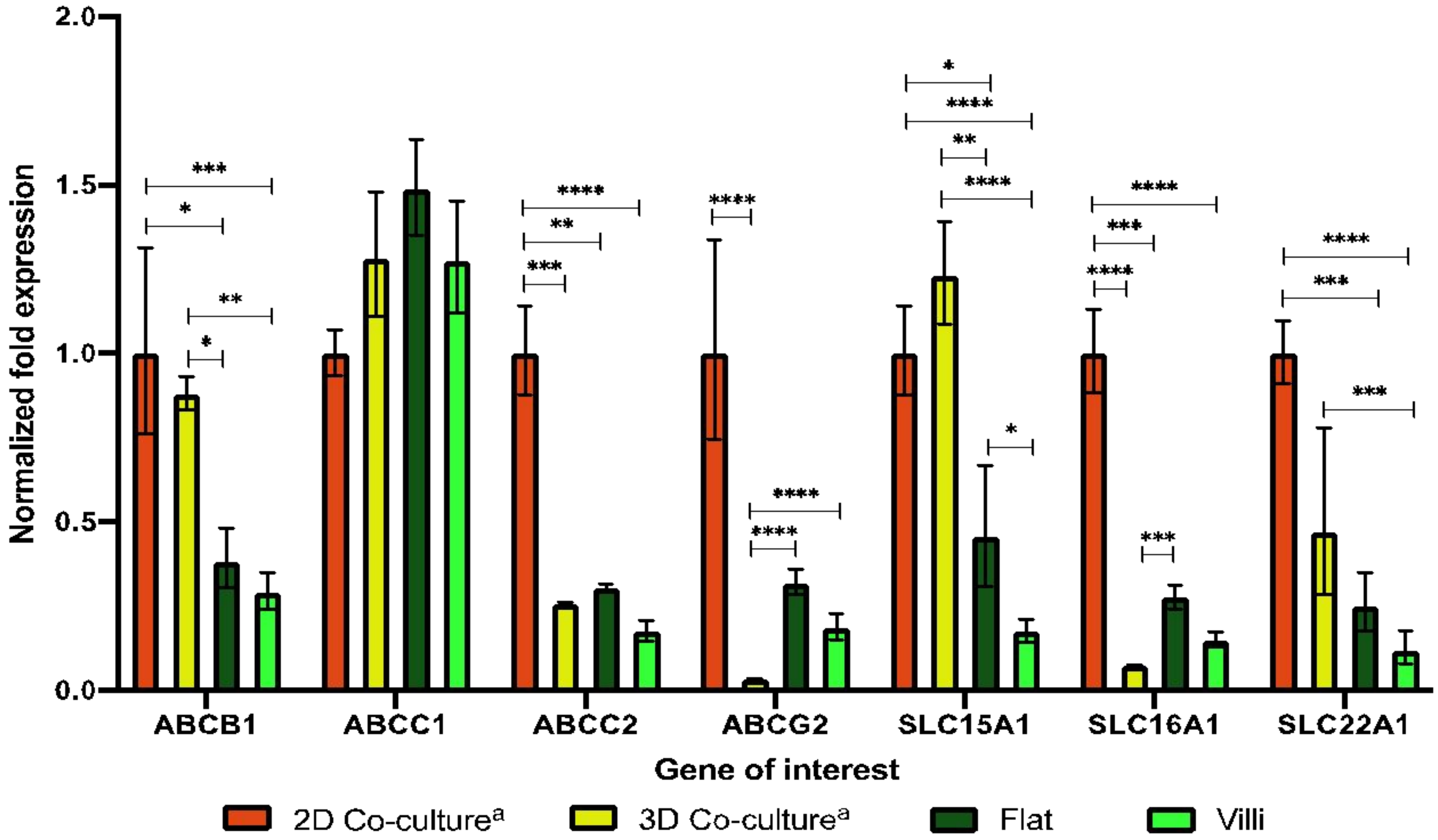


Macedo. et al, under review

A11/00

In vitro intestinal 3D epithelium model

Expression of drug transporters



ABCB1 - P-gp; ABCC1 – MRP1; ABCC2 – MRP2; ABCG2 – BCRP; SLC15A1 – PEPT1
SLC16A1 – MCT1; SLC22A1 - OCT

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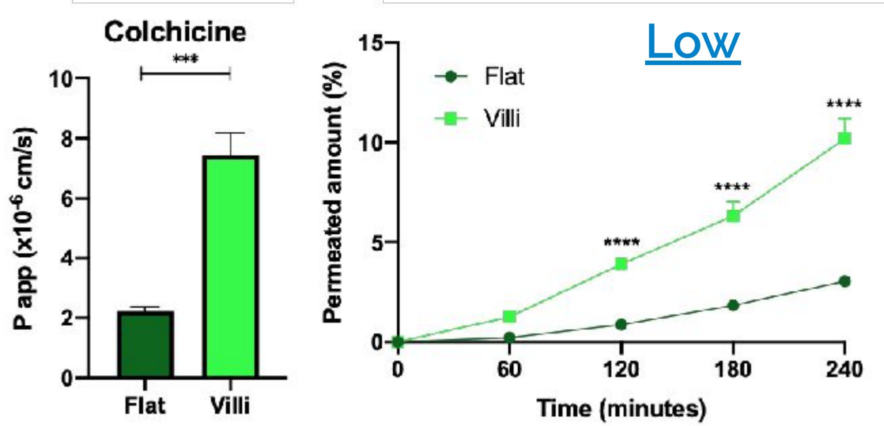

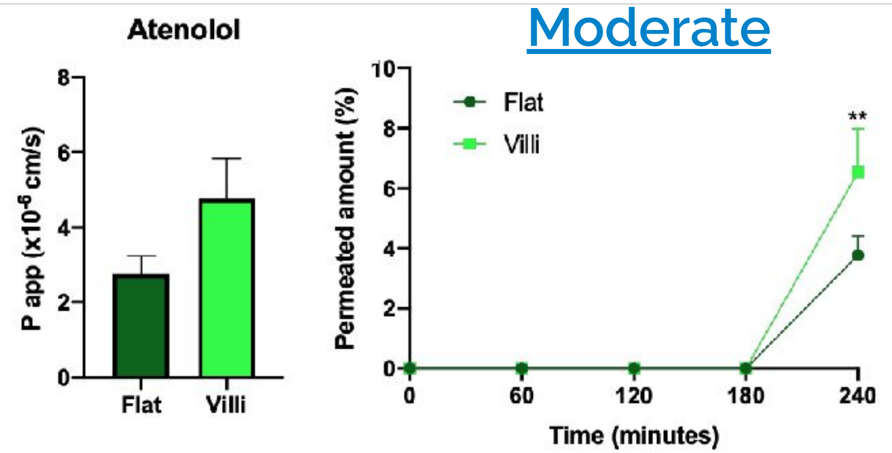

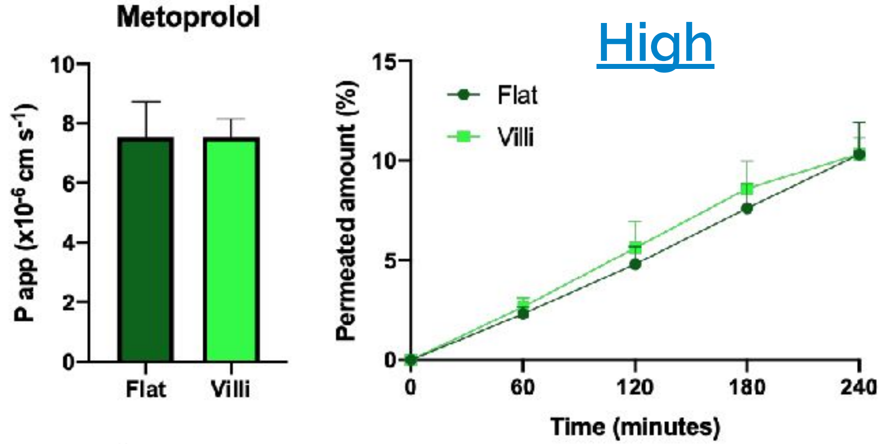

Macedo. et al, under review

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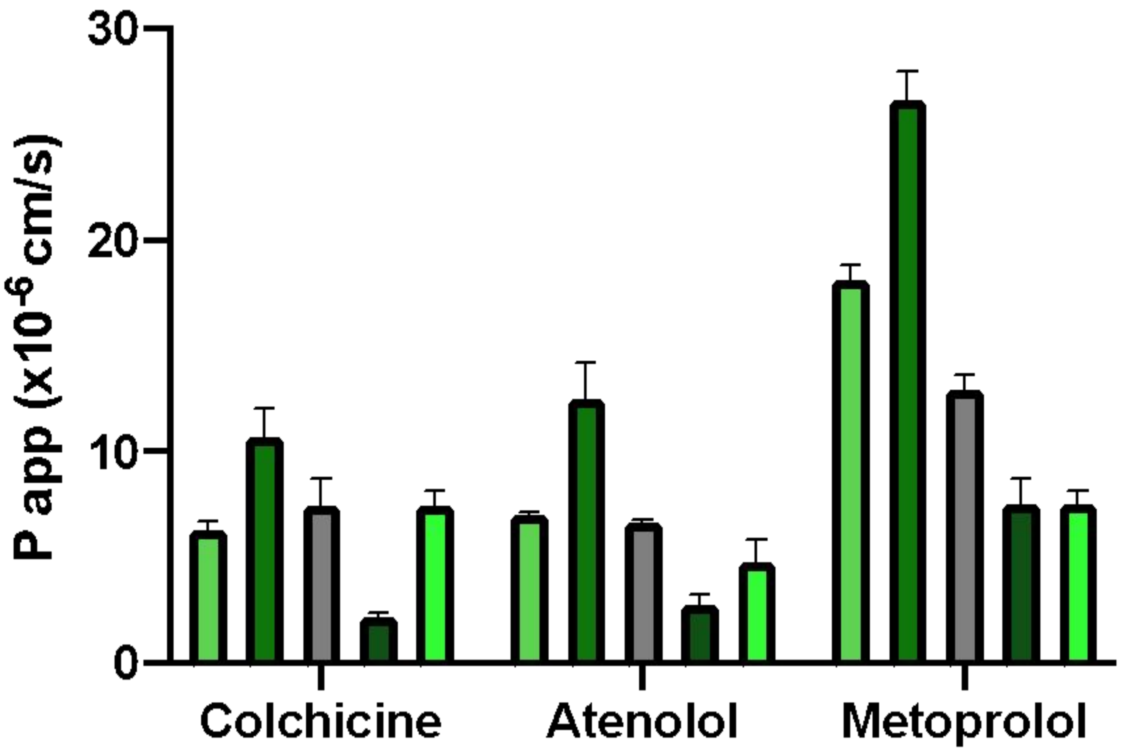
In vitro intestinal 3D epithelium model



F_a 44[1] 50[2] 98[2]

2D Co-culture 3D Co-culture 3D Complete Flat Villi

1. Lozoya-Agullo, I., et al., International Journal of Pharmaceutics, 2017
2. Lozoya-Agullo, I., et al., International Journal of Pharmaceutics, 2015

Macedo. et al, under review

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In summary

- Nanoparticles provide favorable properties for targeted intestinal absorption of anti-diabetic peptides.

- Local intestinal delivery of anti-cancer drugs in nanoparticles to treat colorectal tumor is able to regress tumor evolution

- 3D model of intestinal mucosa mimicking the capillary endothelial layer results in more relevant absorption kinetics of drugs compared to 2D standard model

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Acknowledgements

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PORTUGAL 2020
FCT Fundação para a Ciência e a Tecnologia
CESPU INSTITUTO UNIVERSITÁRIO DE CIÊNCIAS DA SAÚDE
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