THE FUTURE OF PERMEABILITY TESTING

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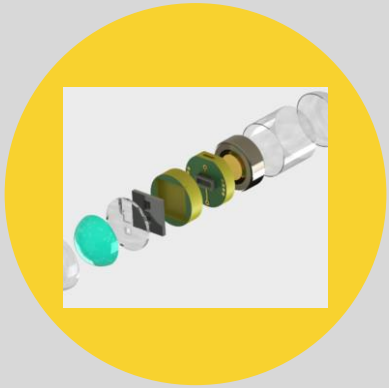
M-CERSI 6th December 2021

The Future of Permeability Measurement

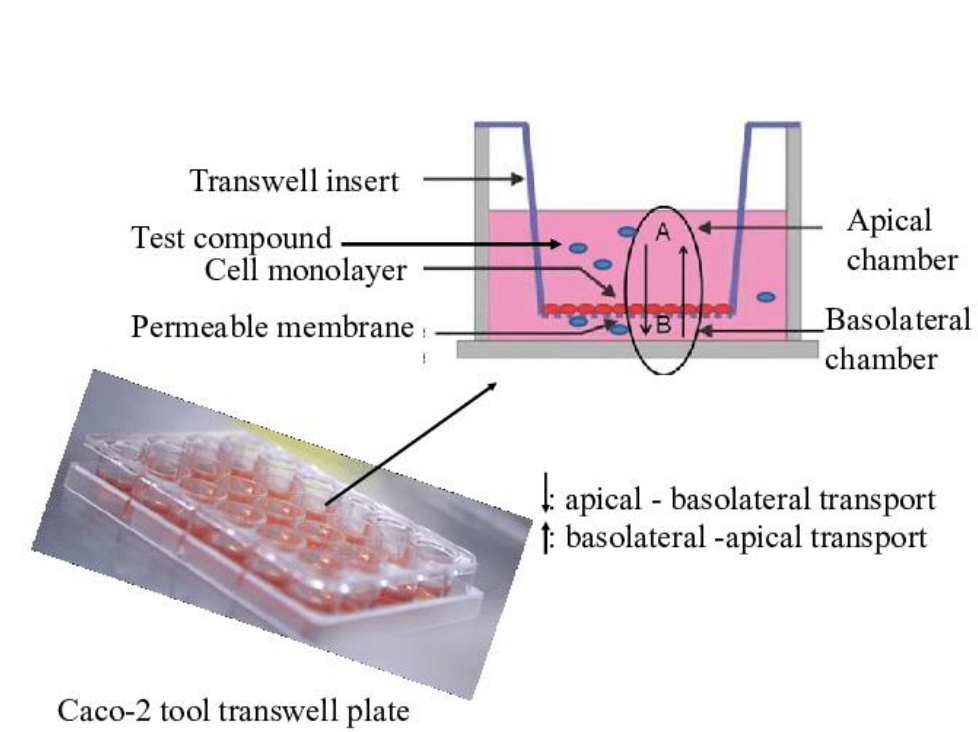
IMPROVING MODEL SET-UPS TO MEASURE PAPP

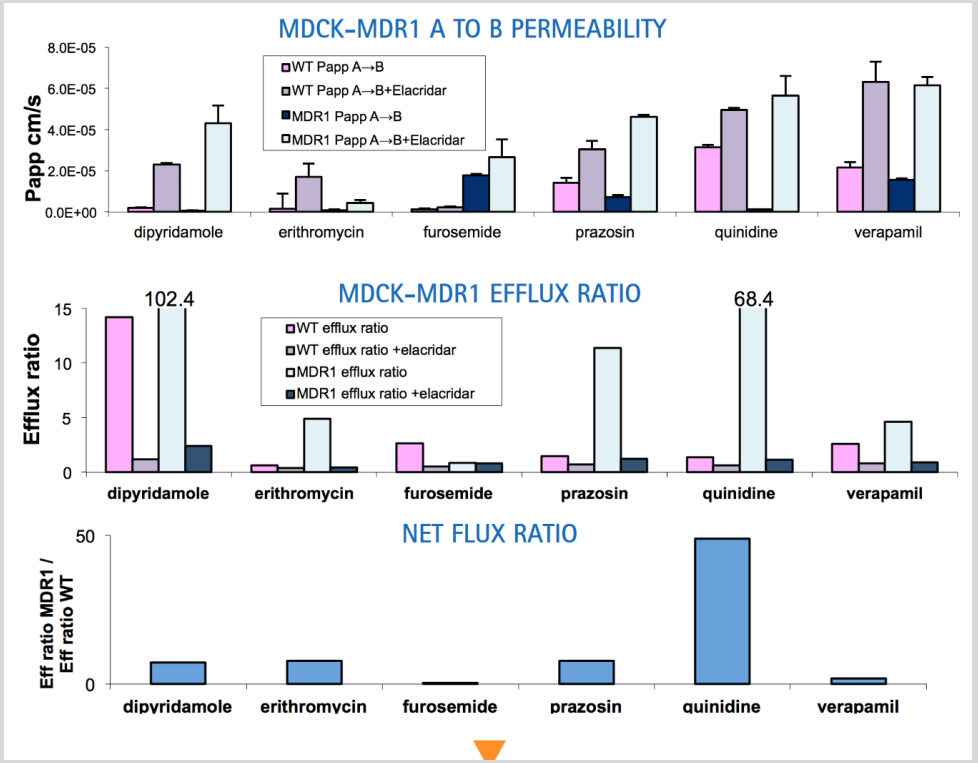
REFINEMENT OF CORRELATIONS BETWEEN EXPERIMENTAL DATA AND HUMAN PEFF



MEASURING PEFF IN HUMANS – QUO VADIS?

Starting point – what do we do now?





Creating better models for the small intestine mucosa

- Over the last several years, there has been growing interest in 3D models of the GI mucosa

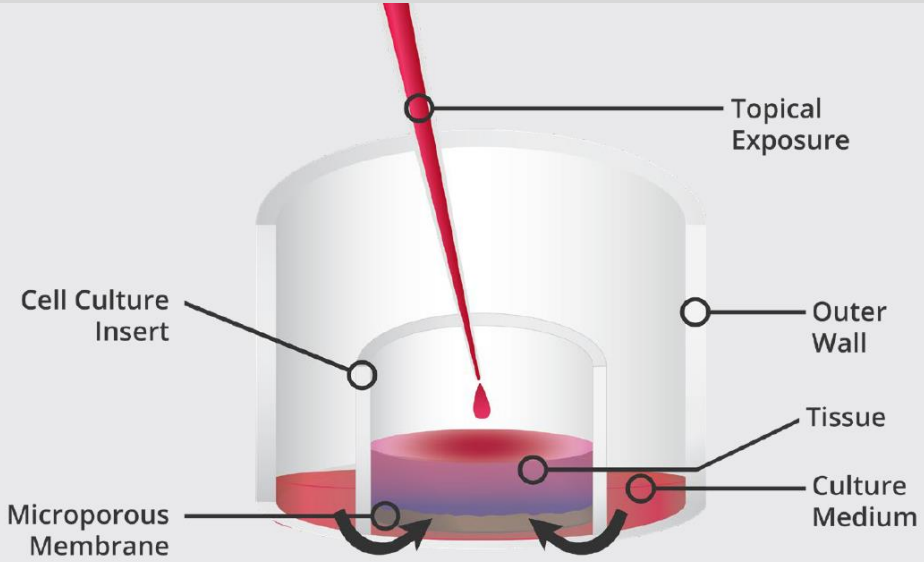
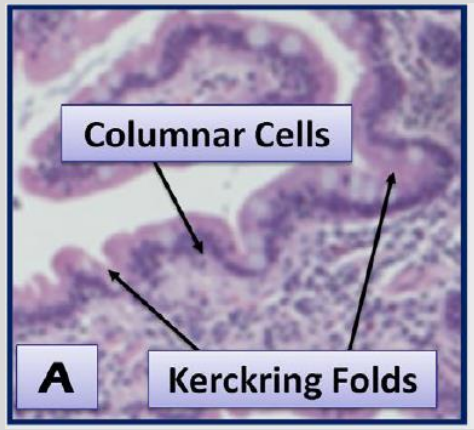
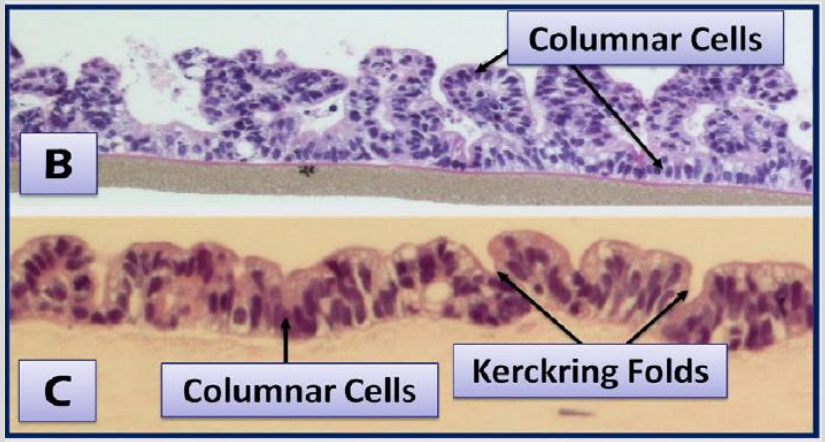


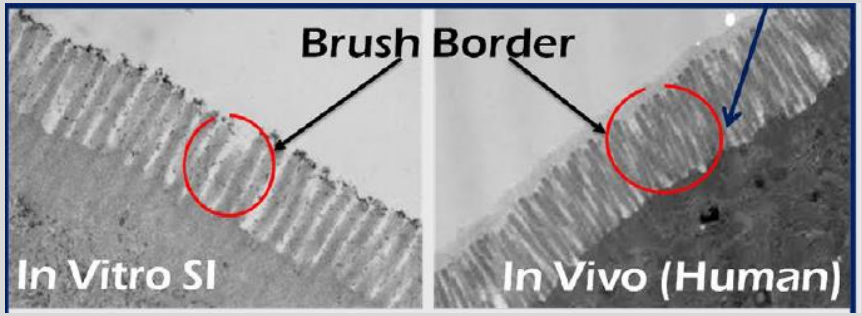
Figure 1: A schematic representation of the SMI tissue model. The tissue is "air-lifted" by culturing it in a microporous-membrane cell culture insert with the apical surface exposed to the atmosphere and is fed by medium diffusing through the membrane. Systemic exposure conditions are achieved by adding the test chemicals into the medium.

- The schematic shows the set up from MatTek, which was presented at AAPS in 2014
- In this approach, small intestinal (SMI) epithelial cells were harvested from post-mortem donors following IRB approval. SMI cells were seeded onto cell culture inserts (partial thickness tissue, SMI-100) or onto a myofibroblast collagen-gel matrix (full-thickness tissue, SMI-100-FT), raised to the air liquid interface and cultured for 2 weeks in specially formulated culture medium designed to induce differentiation.

Creating better models for the small intestine mucosa





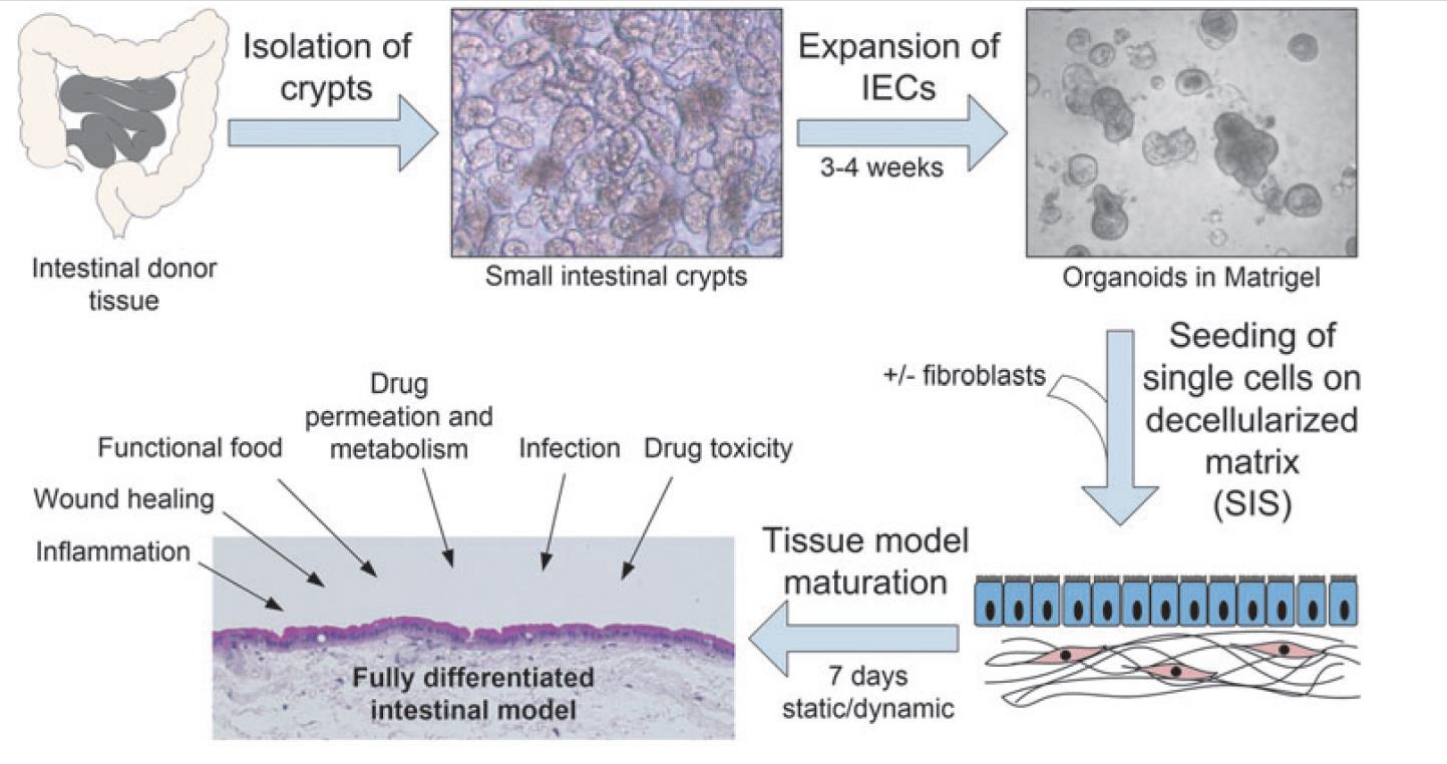


H&E staining showing the: **(A)** epithelium of explant small intestinal tissue and **(B)** partial thickness (PT) and **(C)** full-thickness (FT) EpiIntestinal tissue model. Micrographs of brush border membranes, tight junction formation and mucous secretory granules are also shown.

- Data from Ayehunie et al. AAPS NERDG Annual Meeting 2014 (MatTek.com)

Creating better models for the small intestine mucosa

- Over the last several years, there has been growing interest in 3D models of the GI mucosa



- In this approach, full-thickness jejunal tissues for isolation of human intestinal epithelial cells (hIECs) were obtained from obese patients undergoing a stomach bypass operation at the surgery unit, of the University Hospital Würzburg. The IECs were isolated from human small intestinal tissue and expanded as an organoid culture for 3–4 weeks.
- After enzymatic digestion, single cells were seeded on a decellularized biological scaffold (SIS) with or without intestinal fibroblasts.
- Tissue models were cultured for 7 days under static or dynamic conditions and can be used for various research applications

Creating better models for the small intestine mucosa

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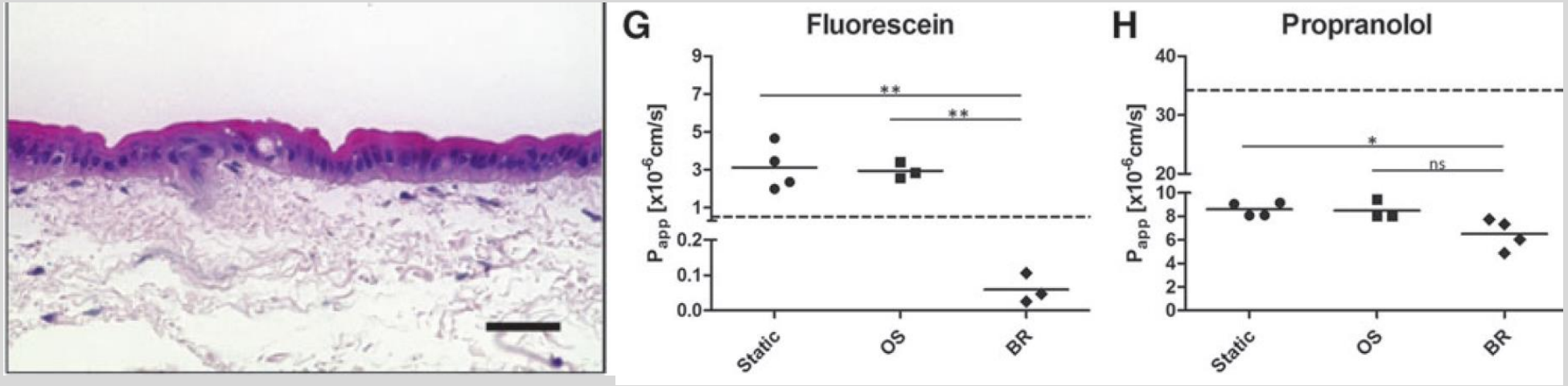
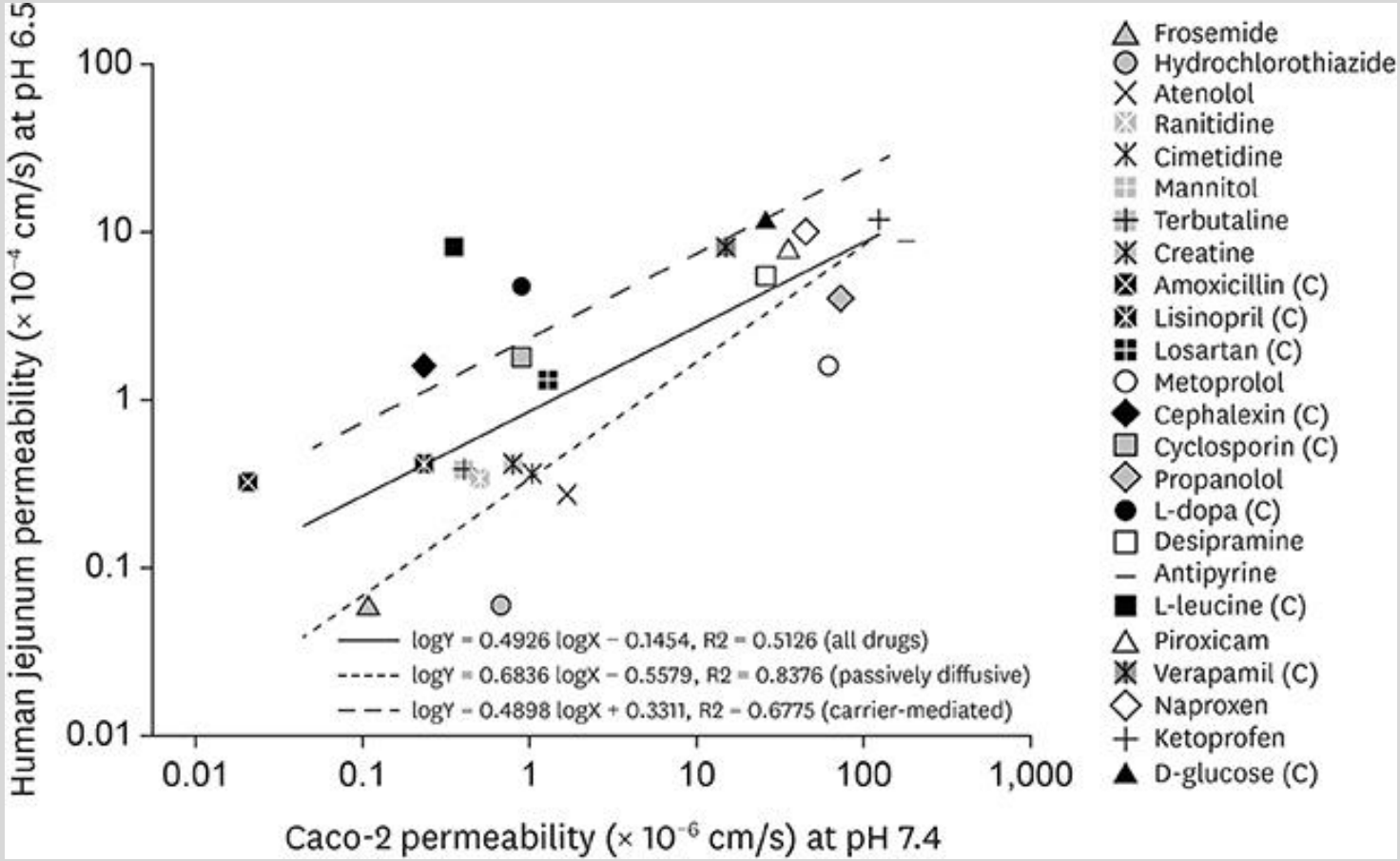


- The left hand side shows the development of the mucosa in a flow bioreactor, while the permeability data are shown on the right hand side for fluorescein, a poorly permeable compound, and propanolol, a highly permeable compound. Comparison of results in a static set up with those in the dynamic environment of bioreactor illustrates the importance of fluid dynamics on the results. All data taken from Schweinlin et al. Tissue Engineering DOI: 10.1089/ten.tec.2016.0101

Correlation of Papp to Peff values - 2002



D. Sun et al. Pharm Res 2002;19:1400–1416.

Correlation of Papp to Human Peff - now

Improvements in the last two decades have been numerous:

- Introducing a pH gradient (pH 6.5 to 7.4)
- Calculating a->b to b->a ratios to evaluate potential for Pgp efflux
- Using biorelevant media as the apical phase (FaSSIF V1)
- Using cell lines with shorter set-up times
- Transferring to a 96-well plate to facilitate more rapid screening
- Transfecting cell lines to create a better simulation of the small intestine mucosa

BUT

- We still don‘t have an immortalized healthy enterocyte cell line
- Methods lack ability to differentiate duodenal / jejunal / ileal / colonic Papp
- Difficult to assess transporter contributions to permeability *vis a vis* passive diffusion

Getting the most out of Papp data

Calculation of average absorption rate from Papp data

ADAM Model Permeation Rate

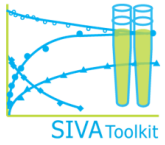
A. Simplest Model

$$V_{n,t}\frac{dC_{Total,n,t}}{dt} = A_{Total,diss,n,t}\cdot\frac{2}{R_{SI,n}}P_{eff,n,t}$$

- Absorption rate from n^{th} ADAM segment
- $V_{n,t}$: Water Volume
- $A_{Total,diss,n,t}$: Total Amount dissolved
- $\frac{2}{R_{SI,n}}$: SA to vol. ratio

Sink assumption for enterocyte concentration

Modelling of in vitro experiments



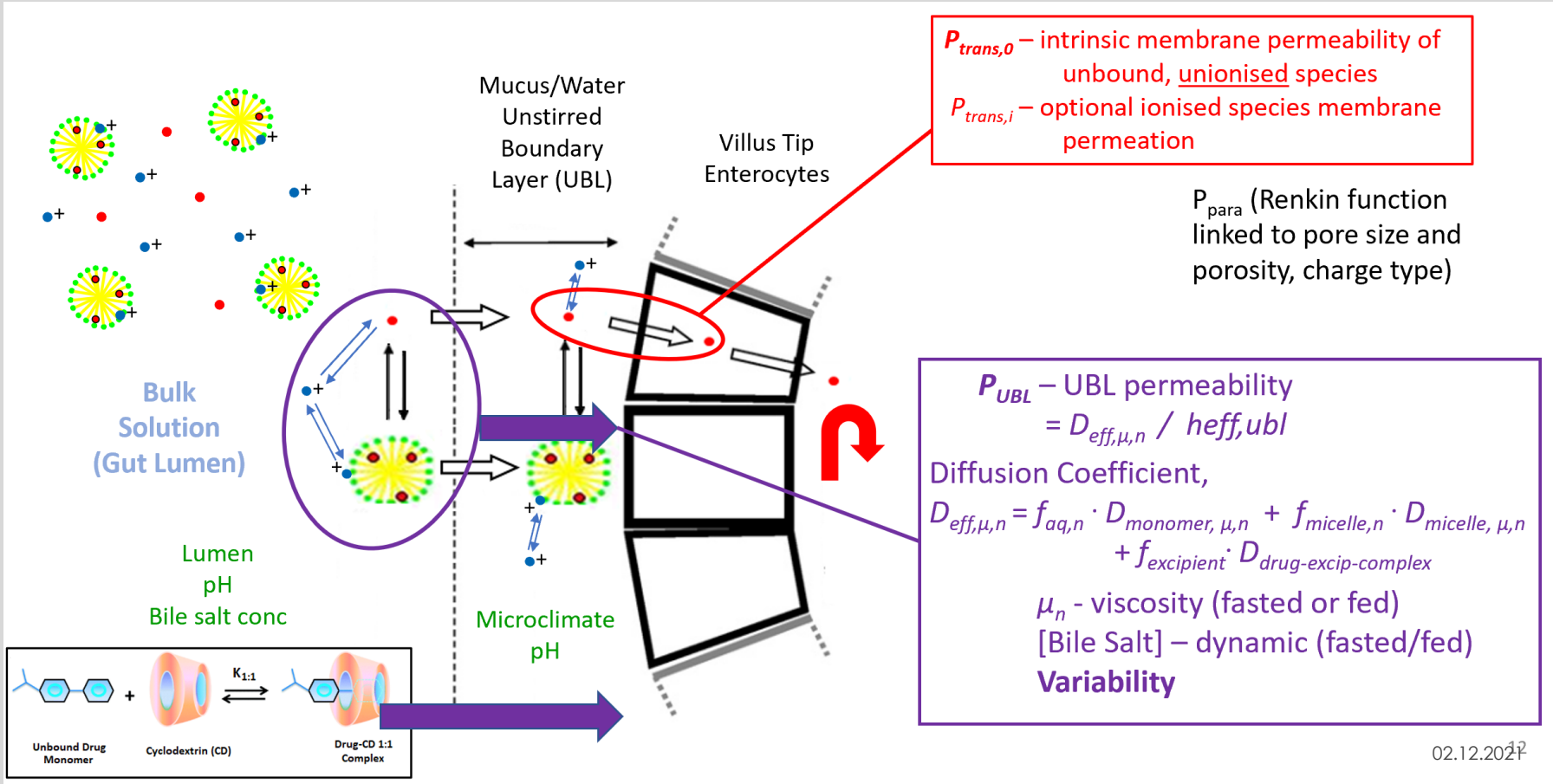
In Vitro Papp

Simple Papp-Peff correlation models
(Peff is proximal jejunum Peff)

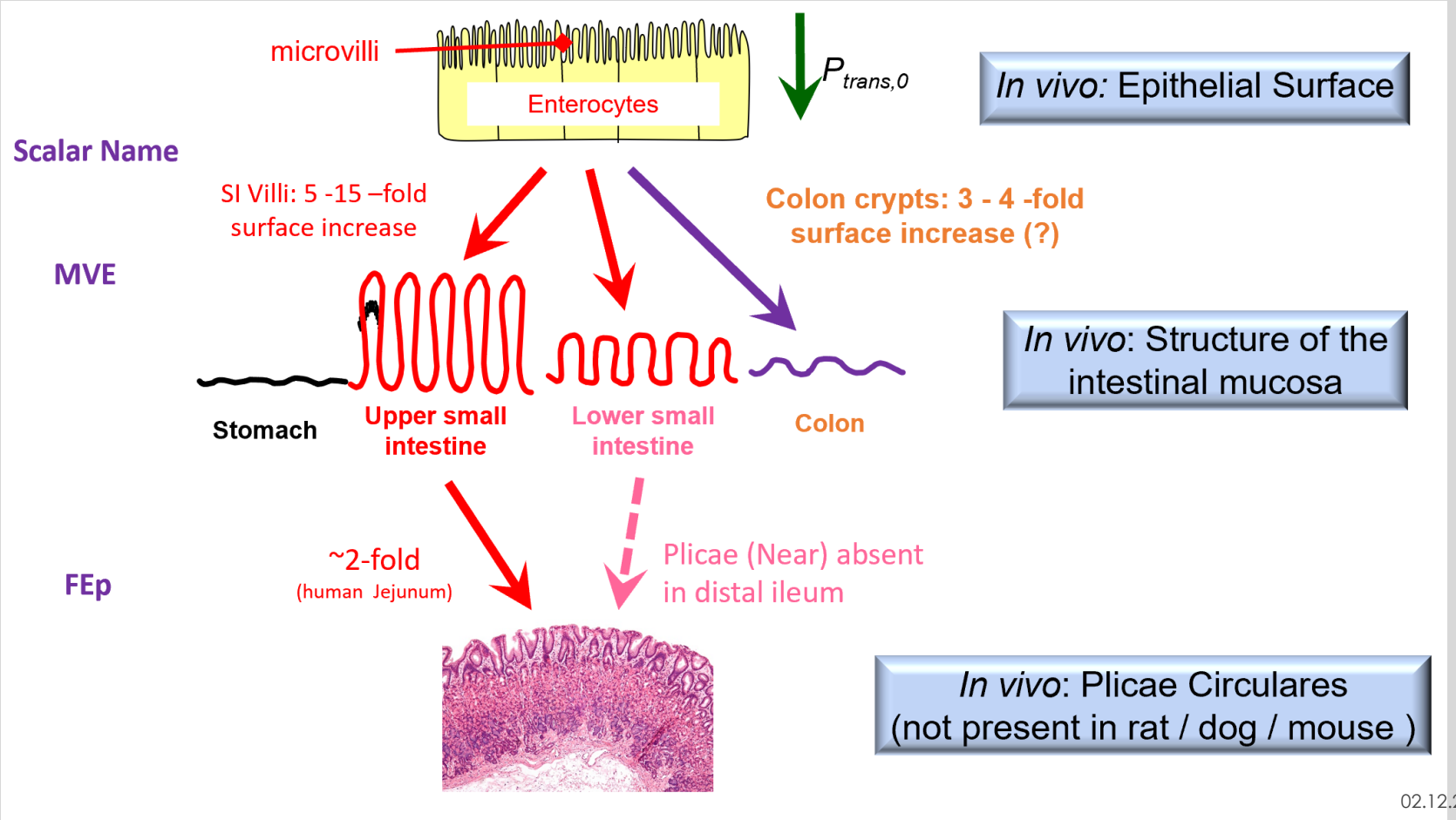
$$P_{eff}\,(10^{-4}\,cm/s) = 10^{A\times\log P_{app}+B}$$

Getting the most out of Papp data

A simple Papp/Peff correlation might suffice, but it would also be useful to create a mechanistic model e.g. when variability among individuals needs to be captured.



Getting the most out of Papp data



Getting the most out of Papp data

B. MechPeff Model*

SA to vol. ratio

Absorption rate from n^{th} ADAM segment

$$V_{n,t}\frac{dC_{Total,n,t}}{dt} = A_{Total,diss,n,t}\cdot\frac{2}{R_{SI,n}}P_{eff,n,t}$$

Water Volume

Total Amount dissolved

Sink assumption for enterocyte concentration

Peff is a composite term

$$P_{eff,n} = \left(\left(\left(P_{Trans,0}\cdot f_{neutral,\boldsymbol{UBL},pH,n} + P_{para,n}\right)\cdot ACC_n\cdot MVE_n\cdot fu_{\boldsymbol{UBL},n}\right)^{-1} + \left(P_{\boldsymbol{UBL},n}\right)^{-1}\right)^{-1}\cdot FEp,n$$

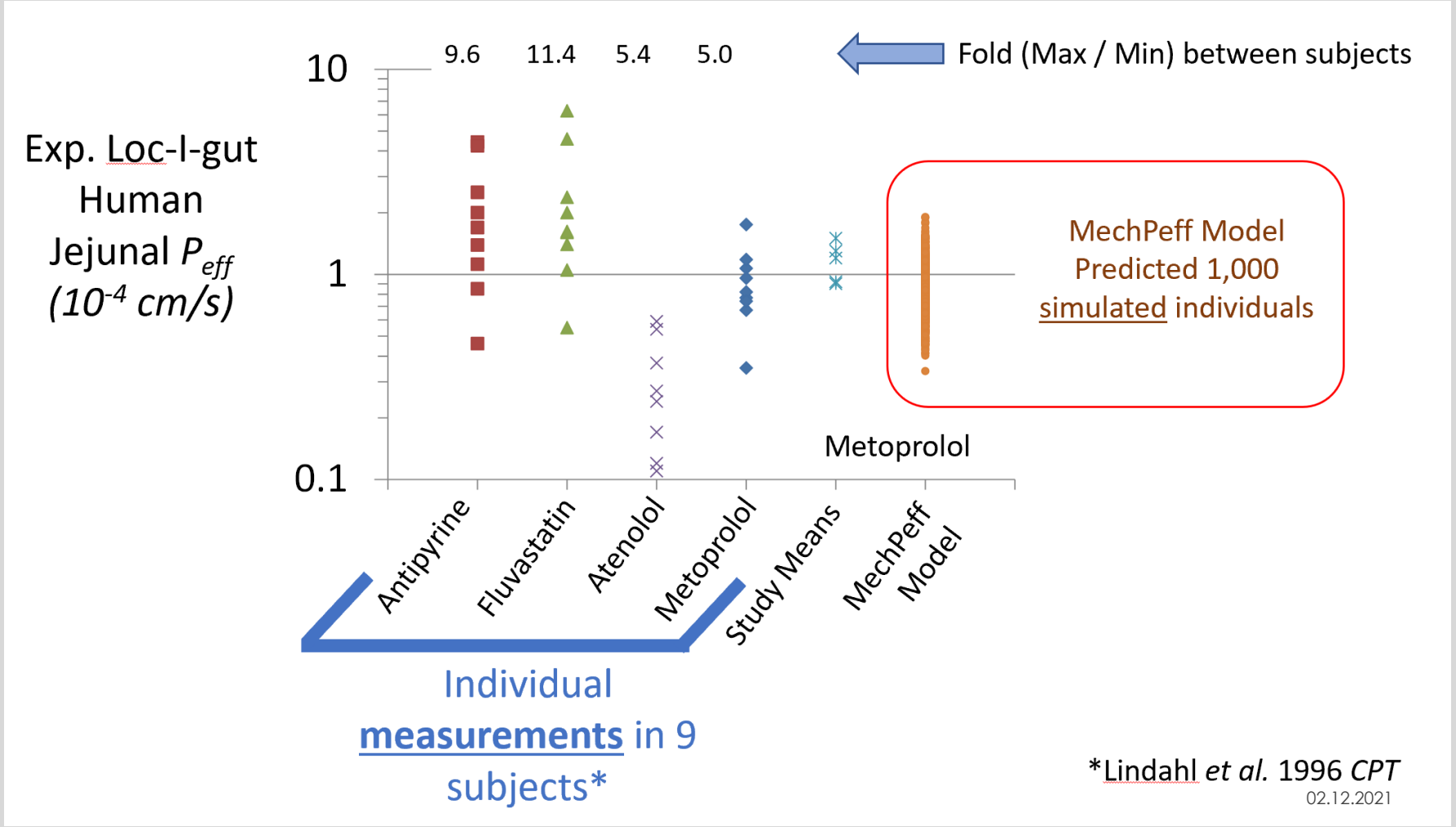
Mechanistic modelling of in vitro experiments (SIVA 4)

Ionisation effects - pH of the UBL)

Free fraction effects linked to bile and/or excipient solubilisation

*Pade et al 2017 *Biopharm Drug Disp.;* (After Sugano 2009)

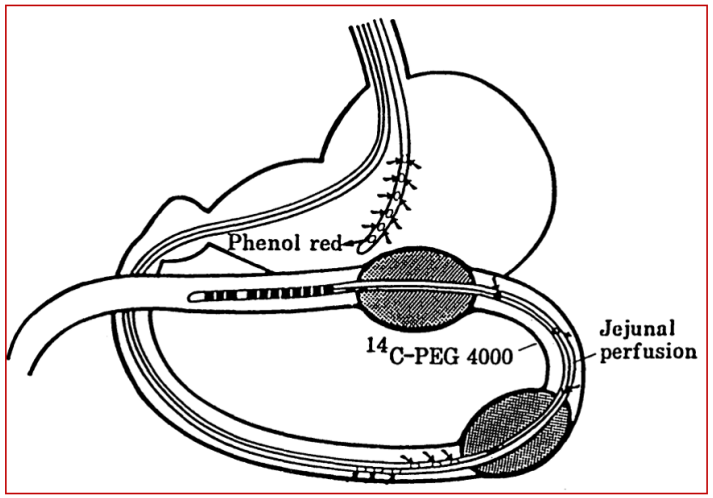
Getting the most out of Papp data



The other side of the equation: establishing Peff in humans

$P_{eff,\ man}$ - effective permeability of the drug in man

***In vivo* measured by a jejunal perfusion system (Loc-I-Gut)**



A **disappearance rate** is measured based on concentrations in the outlet jejunum perfusate (steady state)

A well-mixed tank model is used:

Referenced to cylindrical Surface Area (SA)

$$P_{eff,man} = \frac{C_{in} - C_{out}}{C_{out}} * \frac{Q_{in}}{2\pi rL}$$

Q_{in} = Flow rate of the perfusion solution entering the segment
r = intestinal radius; L= length of jejunal segment
C_{in} = Inlet concentration of drug in the perfusate
C_{out} = Outlet concentration of drug in the perfusate

- Reported **$P_{eff,\ man}$ is in fact nearly always jejunal $P_{eff,man}$**
- The medium for these experiments was usually buffer (non-micellar)

Lennernas *et al.,* 1993

The other side of the equation: establishing Peff in humans

Issues with Loc-i-gut data

- Drug is perfused in a plain buffer solution
- Perfusion rates and volumes are at odds with what we know now about volumes in the small intestine
- Typically only the jejunal Peff is measured – it is difficult to intubate to the ileum or colon
- The disappearance of drug from the lumen is measured – does this always equate with absorption?
- Subjects may be uncomfortable due to the intubation procedure and the tube position has to be checked by fluoroscopy, creating risks for the subject

Tracking Peff as a function of location is easier with a „permeability capsule“

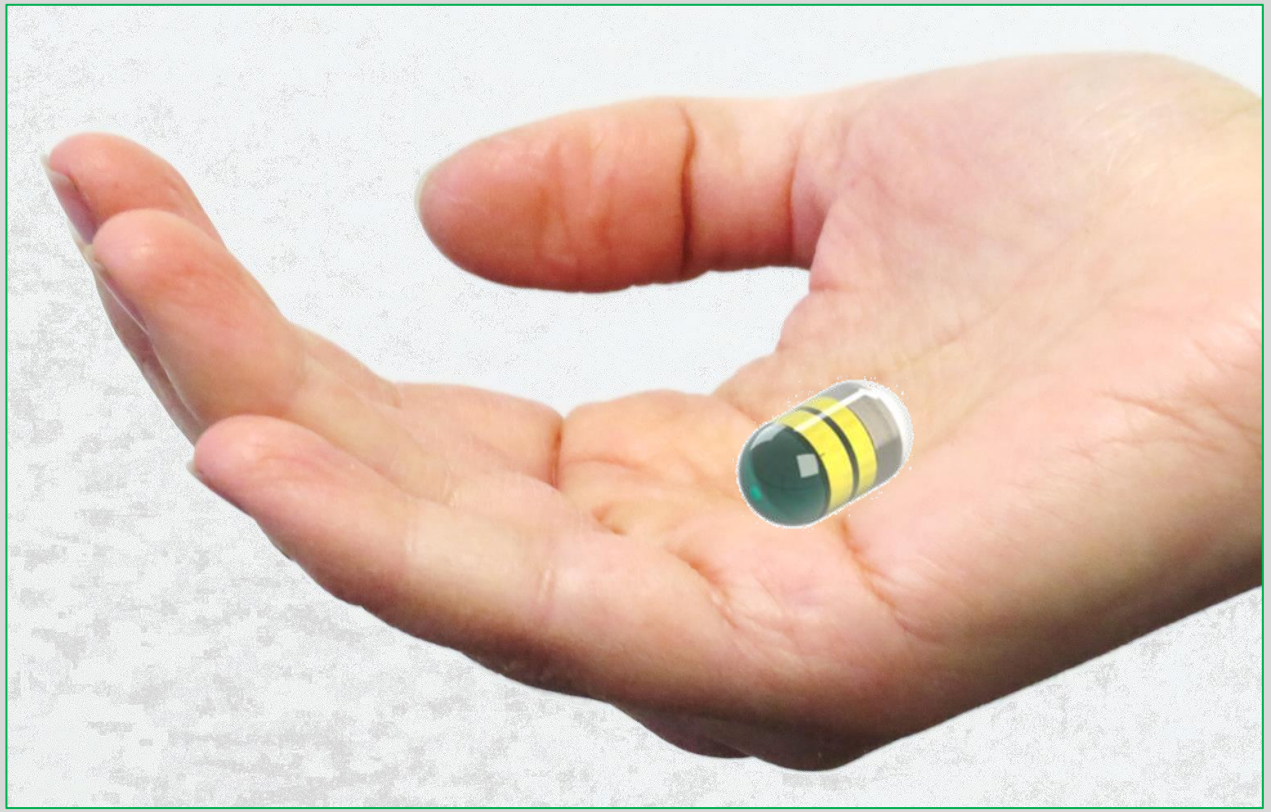
- Söderlind et al. J Contr. Rel. 2015, 217: 300-307 (Metoprolol)

Electronic Pill currently in development at the Fraunhofer Institutes in Germany

The „e-Pille“ project promises

Precise Drug Delivery using easy to swallow, sustainable microdosing devices

Fraunhofer IME Fraunhofer EMFT Fraunhofer IZM

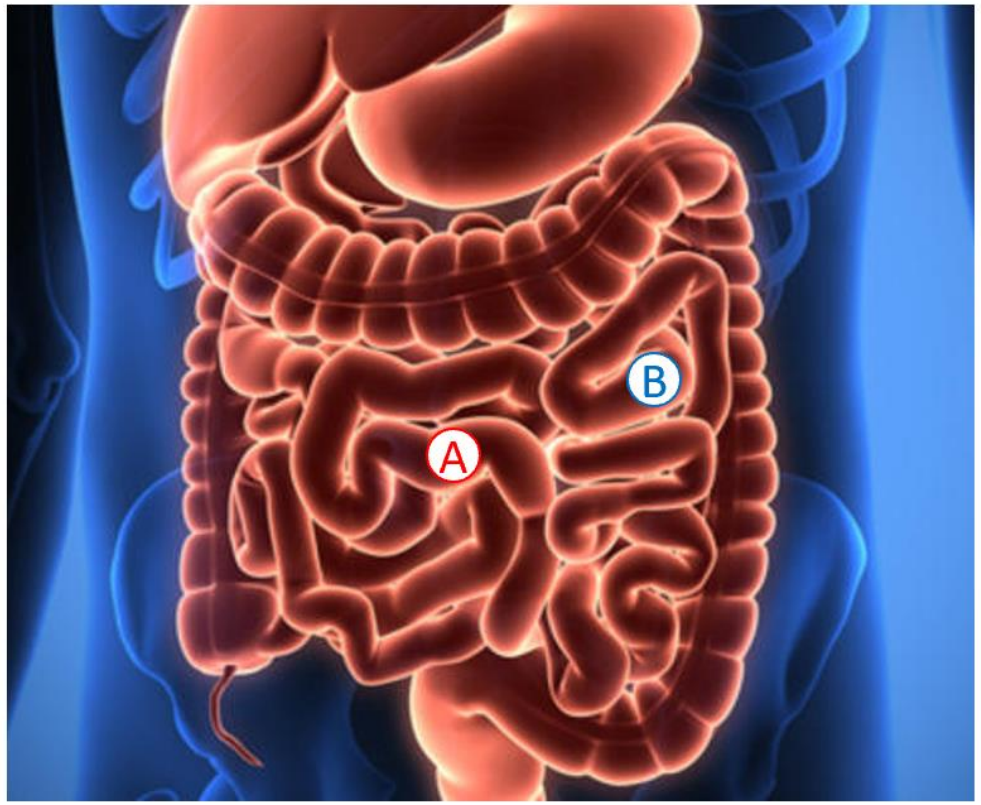


02.12.2021

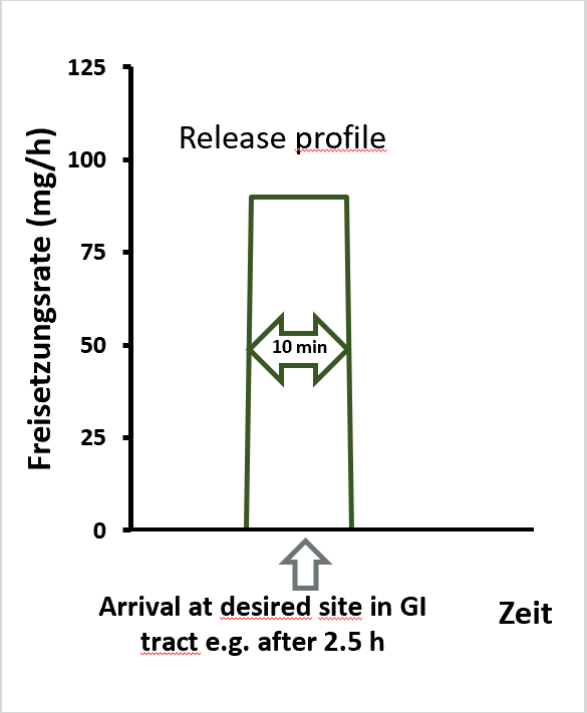
The „e-Pille“

- Will have a modular design
- Be easy to swallow
- Its position in the GI tract can be followed using sensors
- Release can be triggered once the e-Pille has reached the desired location
- Release can be short (10-15 min) to measure permeability at a single location
- Or, longer (over hours) to simulate a controlled release profile

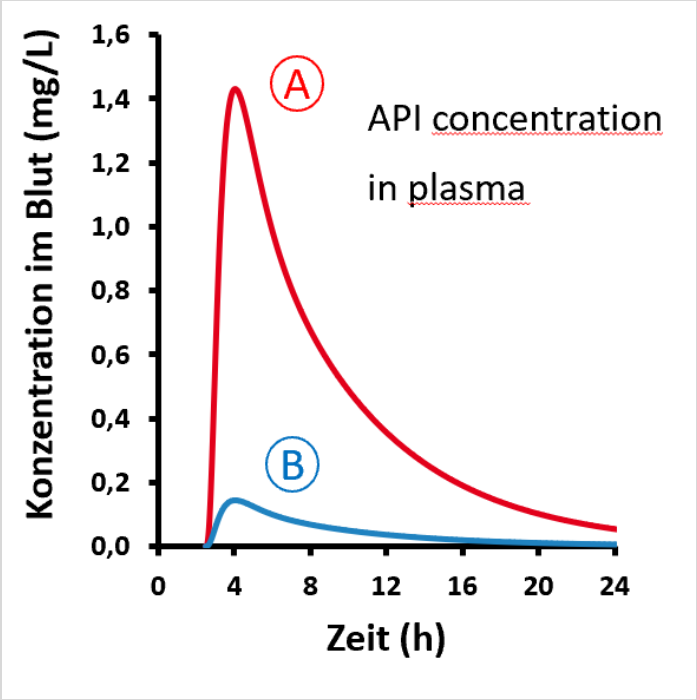
1. The e-Pille is programmed to release the API at a pre-selected location in the intestines (site A or B in this example)



The „e-Pille“



2. The e-Pille releases the API at a constant rate over e.g. 10 minutes at the desired site



3. If, for example, the API is better absorbed from site A than site B, this will be reflected in a higher plasma concentration

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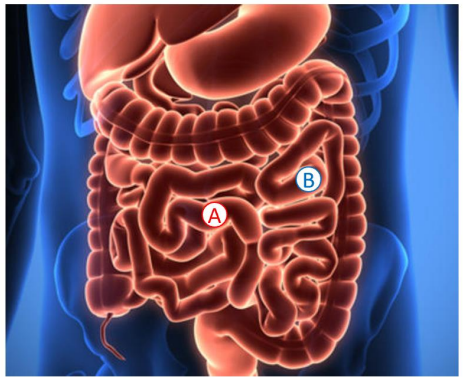
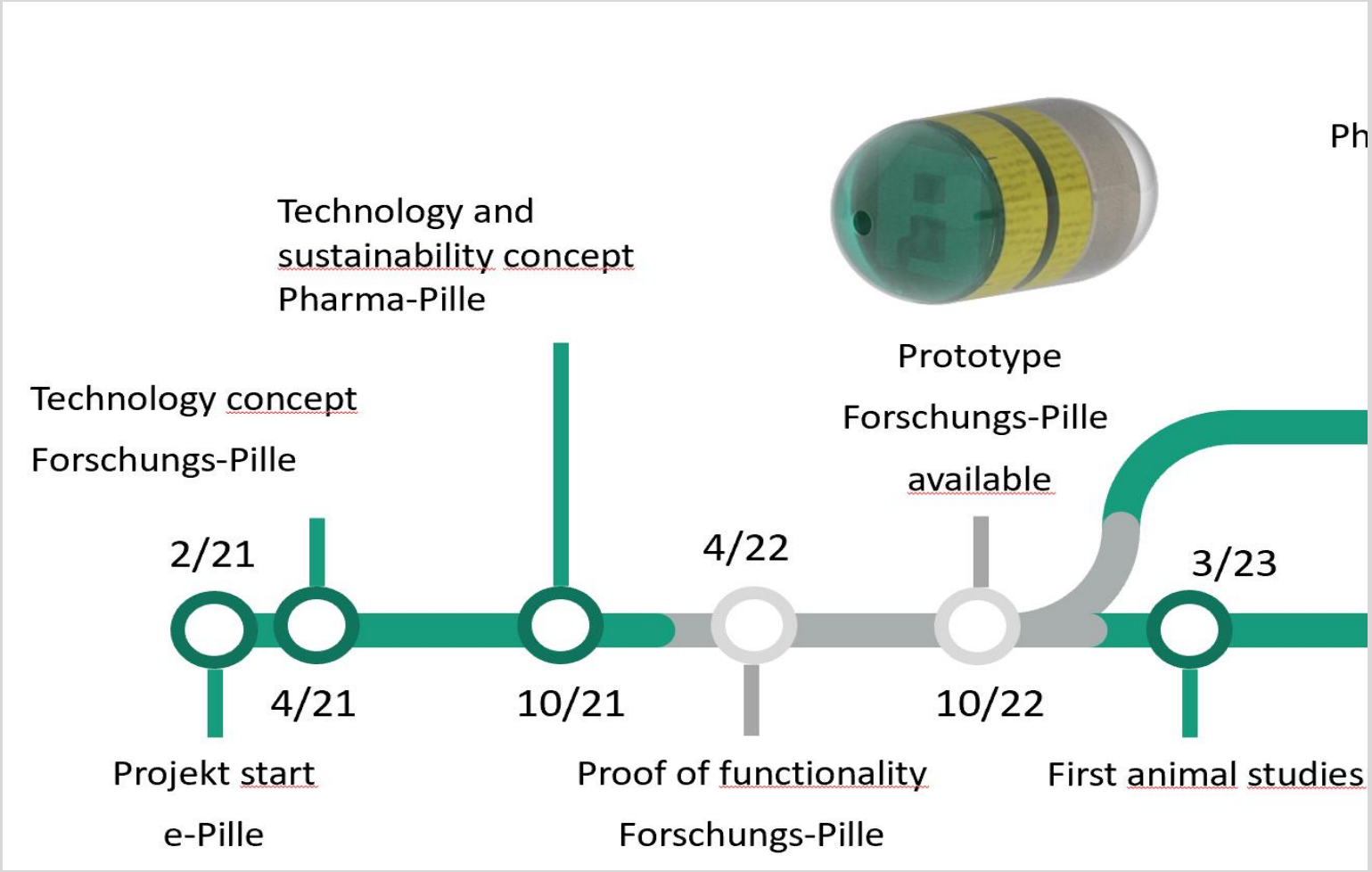


Timeline for the „e-Pille“



Acknowledgments

David Turner

CERTARA

Simcyp

My colleagues at Fraunhofer:

Martin Richter
Sebastian Kibler
Andreas Ostmann
Manuel Seckel
Christopher Frey
Lutz Stobbe
And our team of co-workers at EMFT, IZM and ITMP

Fraunhofer IME

Fraunhofer EMFT

Fraunhofer IZM

Greetings from…..

……and many thanks for your attention!