Developing body-on-chip technology for the reduction and replacement of small animals in early drug discovery PK/PD assessments

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Disclosure: This presentation covers protected intellectual property, UK patent no PG450503GB

Overview

- Background
- Hypothesis and aims
- Designing and testing a novel device
- Optimising co-culture
- Kinetic studies
- Future work

Why?

- 12 years, $1.3bn per drug
- 25% preclinical success rate (n= 449)
- 7.6% likelihood of approval (n= 3496)

Wouters, et al. *JAMA*, *323*(9), 844–853. (2020). https://doi.org/10.1001/jama.2020.1166
Takebe et al. *Clinical and translational science*, *11*(6), 597–606. (2018). https://doi.org/10.1111/cts.12577
Hay et al*. Nat Biotechnol* **32,** 40–51 (2014). https://doi.org/10.1038/nbt.2786

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ALURES – ANIMAL USE REPORTING - EU SYSTEM - SECTION 2. 2020, European Commision.

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Clear need for better early predictors of *in vivo* success

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Clear need for better early predictors of *in vivo* success

- Animal testing of cosmetic products/ingredients banned in EU since 2013
- Push to develop *in vitro*, animal free systems for use in cosmetic product and ingredient safety risk assessments

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Positron emission tomography (PET) - what & why?

- High resolution imaging technique utilising a radiotracer
- Short half-life isotopes ^{18}F (~109min), ^{68}Ga (~68min), and ^{11}C (~20min)
- Combined with CT for structural relevance

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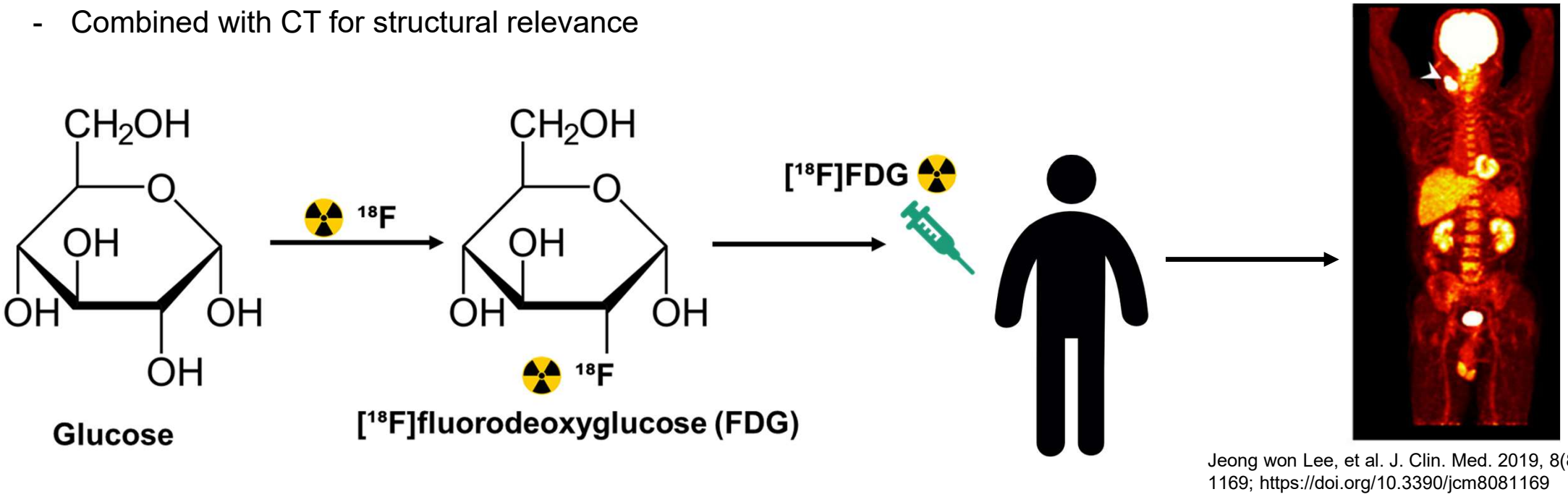

Jeong won Lee, et al. J. Clin. Med. 2019, 8(8), 1169; https://doi.org/10.3390/jcm8081169

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Hypothesis

Body-on-chip platforms capable of circulating drug loaded media across multiple organ compartments can provide PK/PD predictions consistent with that of gold standard *in vivo* human PET data for the same drug.

Aims

- **Optimise the use of a body-on-chip platform such that it is capable of circulating drug-loaded media across multiple “organ” compartments arranged to mimic human physiology.**
- **Use optimised device to sample “organ” drug concentrations at multiple time points for kinetic modelling**
- **Compare kinetic parameters to *in vivo* outcomes in human PET studies of the same compound**

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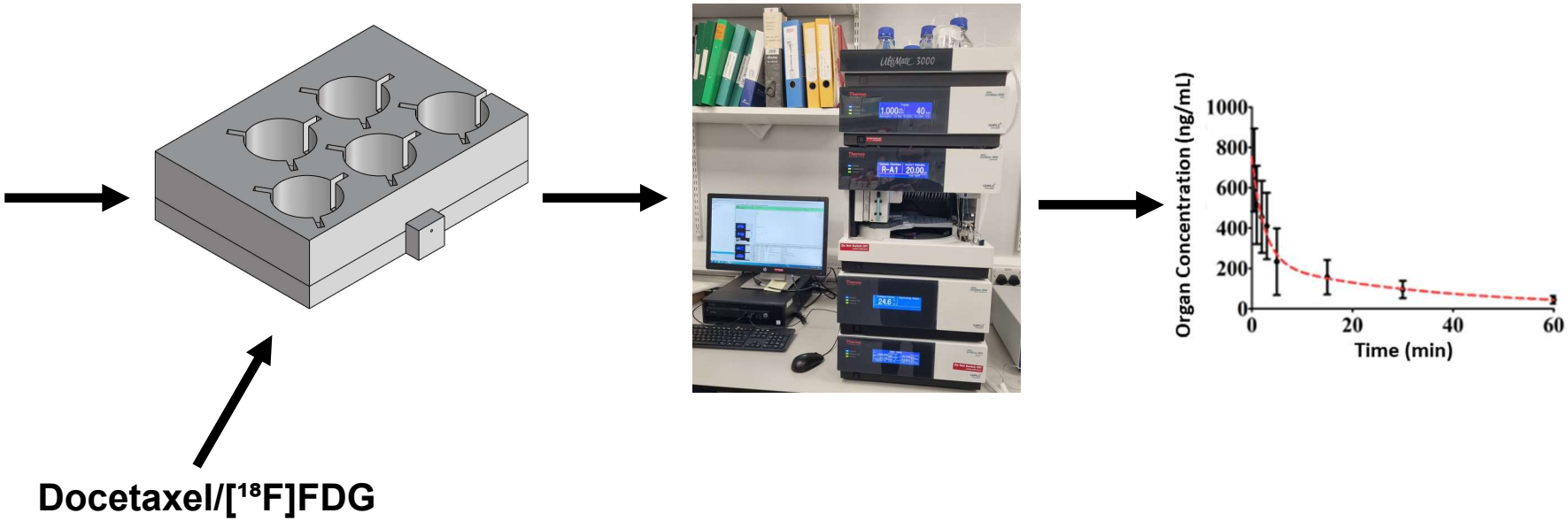
Brain = human neurons (SH-SY5Y)

Lung = human primary bronchial epithelial cells

Liver = hepatocyte cell line (HepG2)

Heart = human primary cardiomyocytes

Kidney= Immortalised RPTECs (SA7K)



Stadulytė *et al.* Journal of chromatography. *B*. 1118-1119, p33-39; (2019). DOI: https://doi.org/10.1016/j.jchromb.2019.04.026

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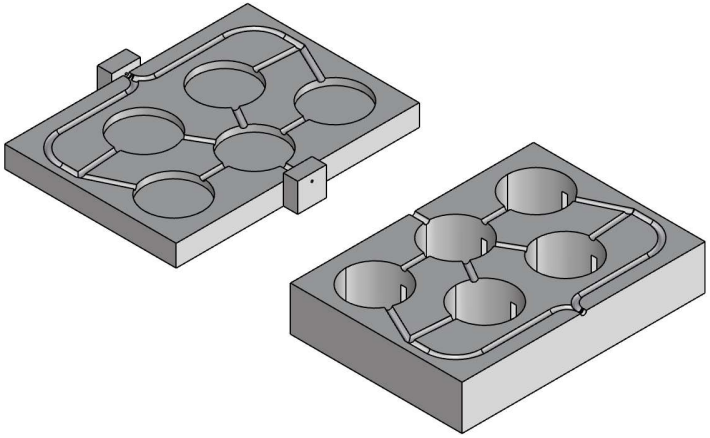
Design & test body-on-chip device

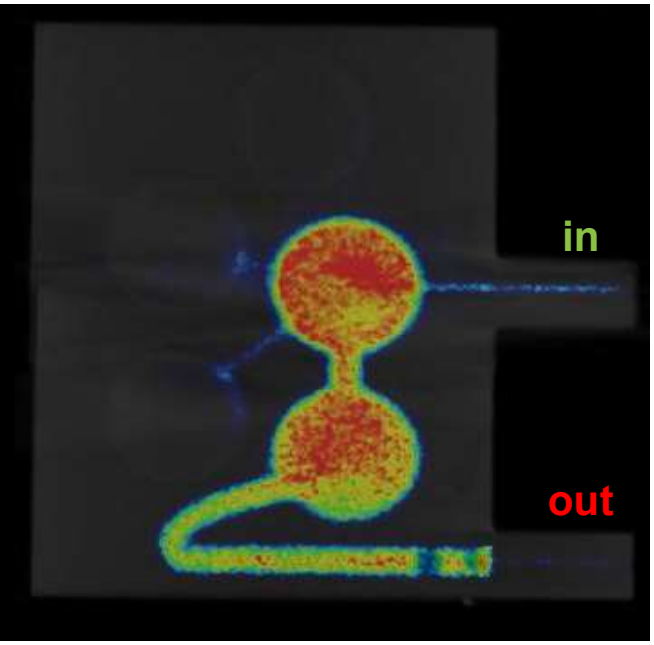
THE UNIVERSITY of EDINBURGH
Edinburgh College of Art

Produced 3D printed prototypes



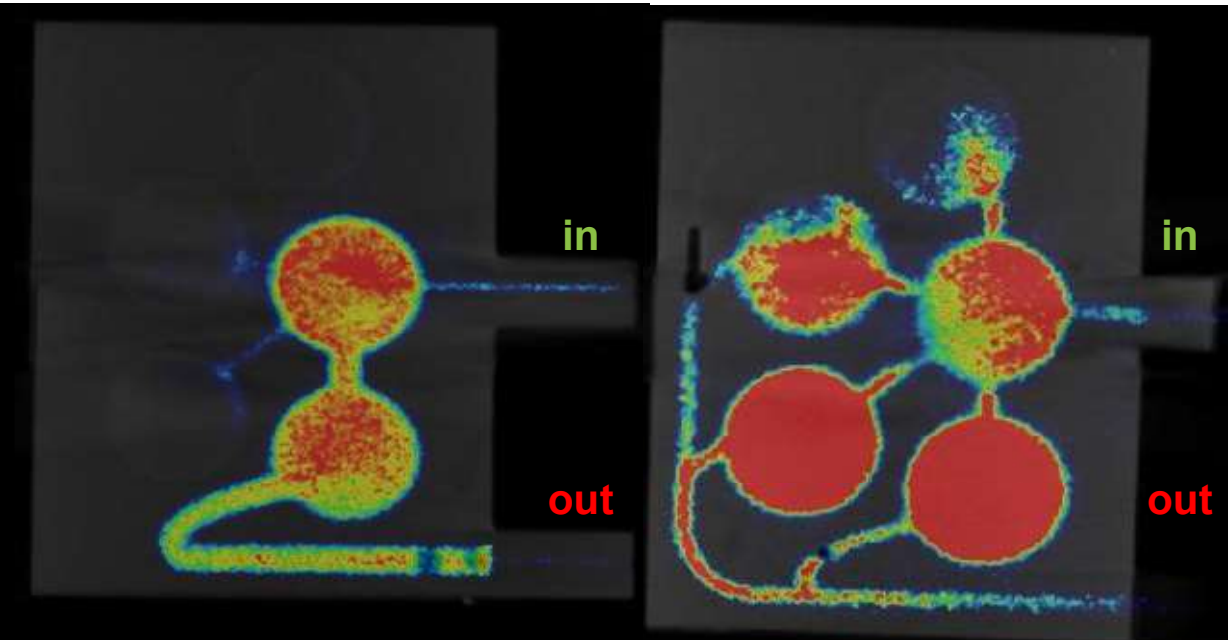
[^{18}F]FDG/[^{18}F]NaF PET scans to assess flow

Design & test body-on-chip device



Capillaries scaled to *in vivo* blood flow:organ volume ratio

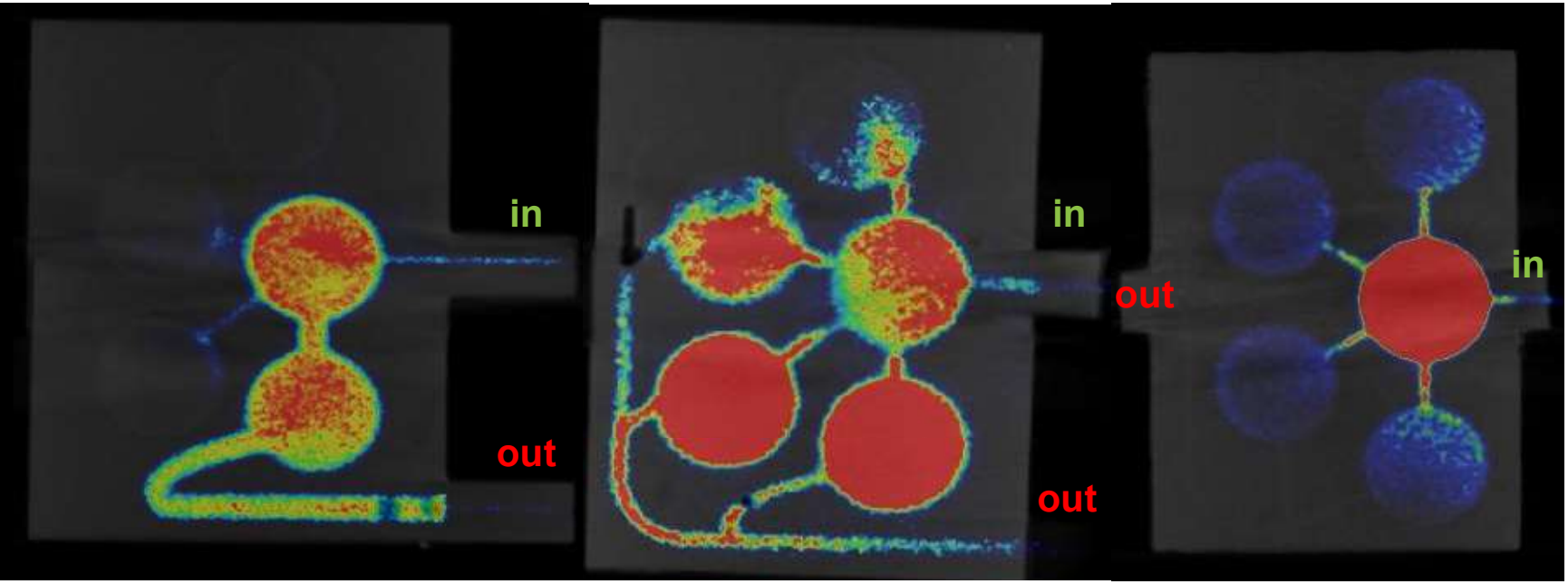
Design & test body-on-chip device



Capillaries scaled to *in vivo* blood flow:organ volume ratio

Capillaries set to same size (2mm)

Design & test body-on-chip device

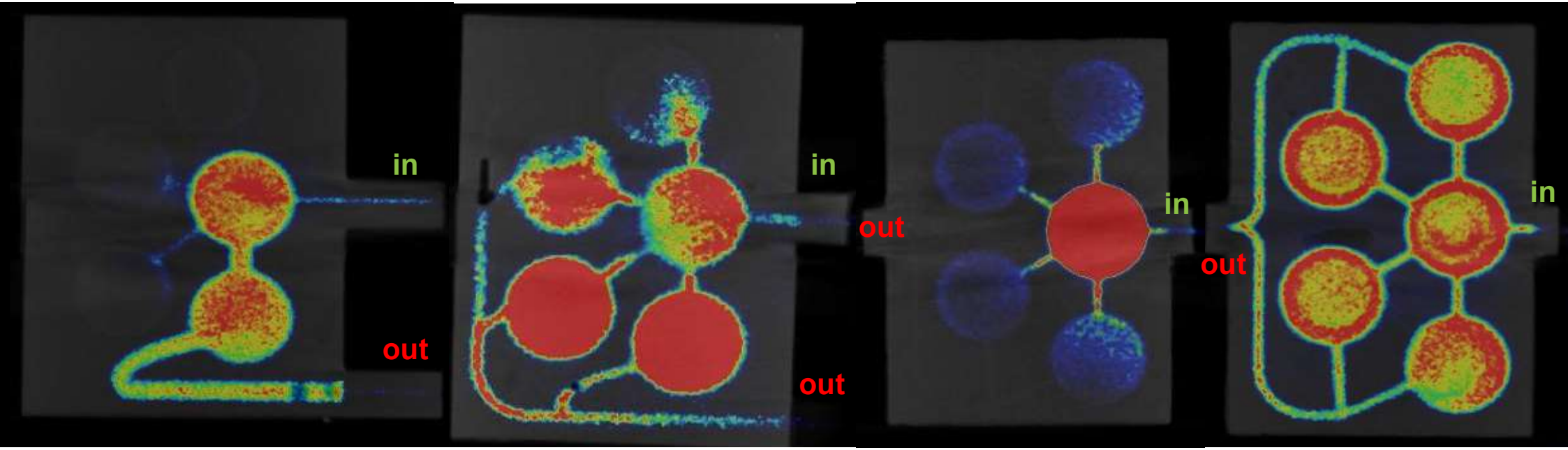


Capillaries scaled to *in vivo* blood flow:organ volume ratio

Capillaries set to same size (2mm)

Capillaries same size + completely symmetrical

Design & test body-on-chip device



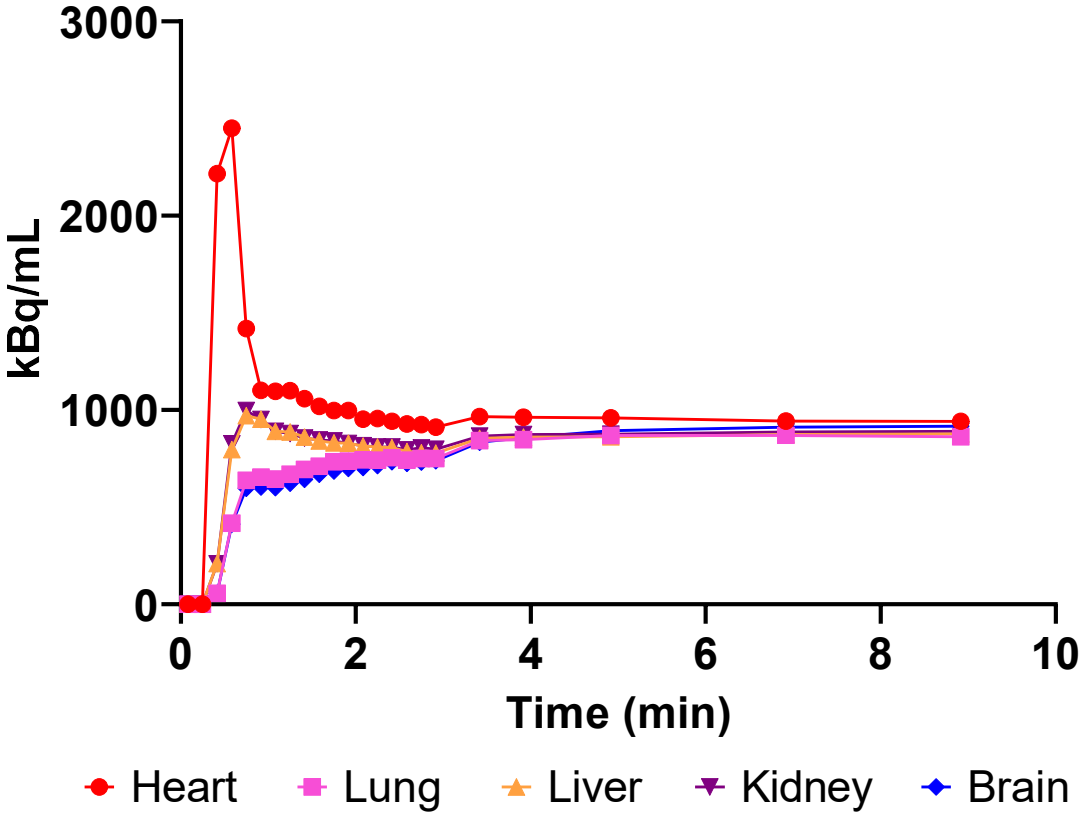
Capillaries scaled to *in vivo* blood flow:organ volume ratio

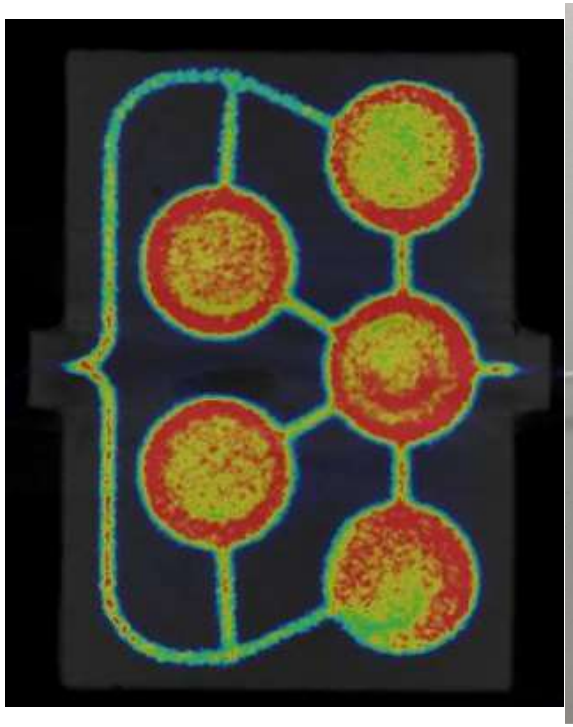
Capillaries set to same size (2mm)

Capillaries same size + completely symmetrical

Capillaries same size + completely symmetrical, with optimised flow rate

Design & test body-on-chip device





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Cell culture media optimisation

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Common medium?

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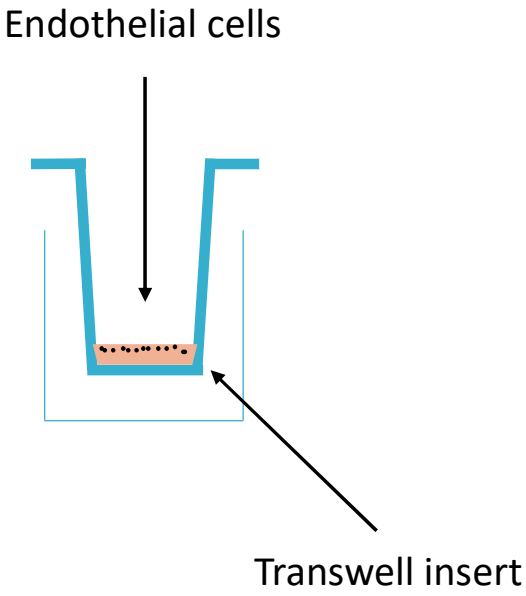
~~Common medium?~~

Separation of compartments

Separation of compartments via endothelial barrier



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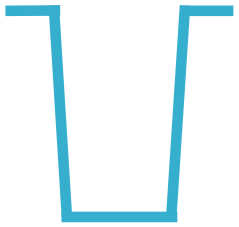

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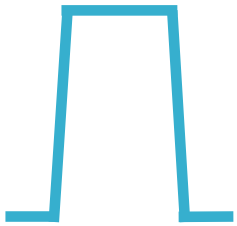

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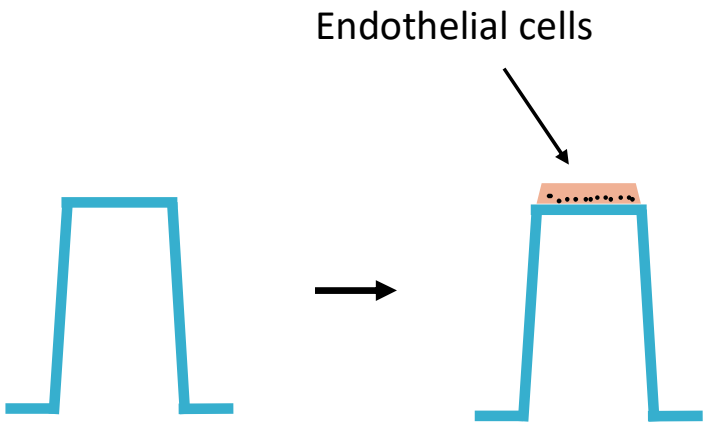

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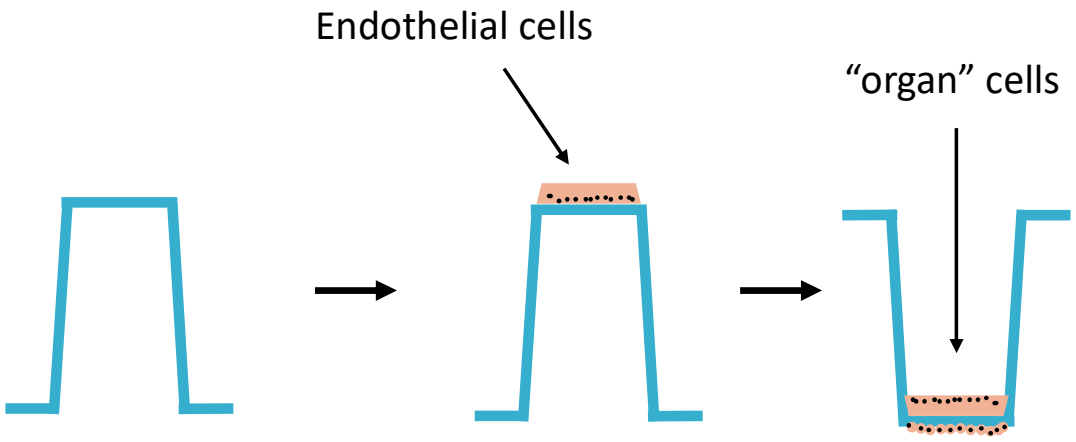

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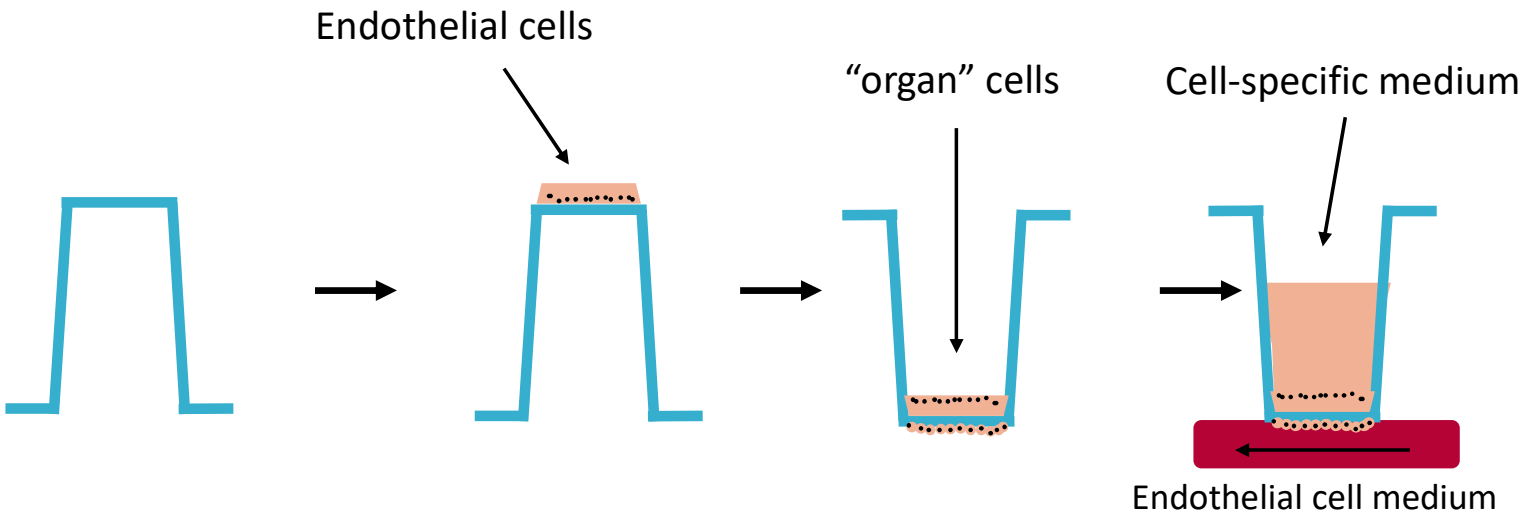

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Modelling definitions

K_i = the rate of influx for a model using irreversible binding (Patlak model for FDG)
V_T = Total volume of distribution
SUV = standardised uptake value, calculated as concentration in tissue normalised to injected dose and body weight
SUVmean = the average SUV across a tissue/organ of interest

In vitro FDG K_i significantly correlates with *in vivo* SUVmean

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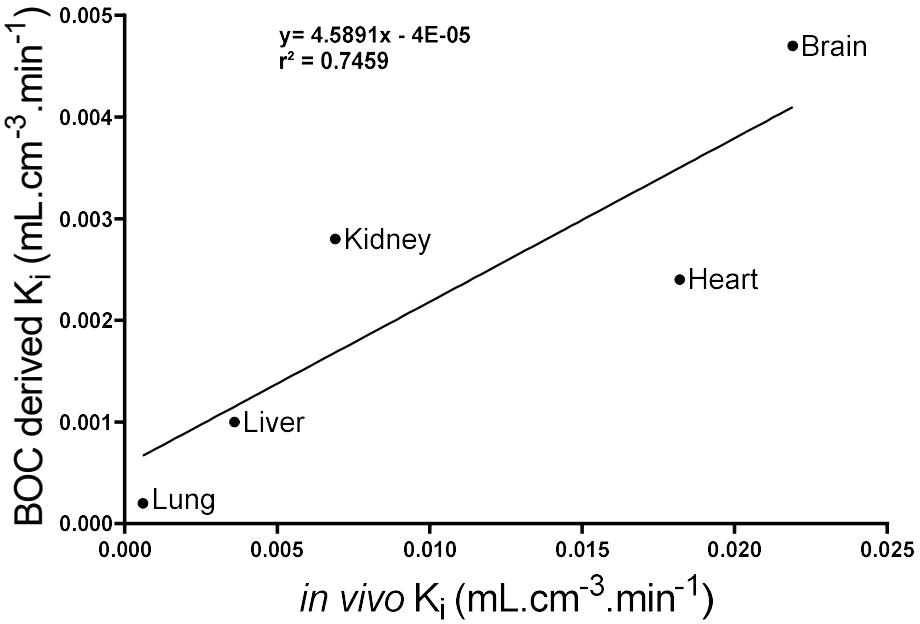
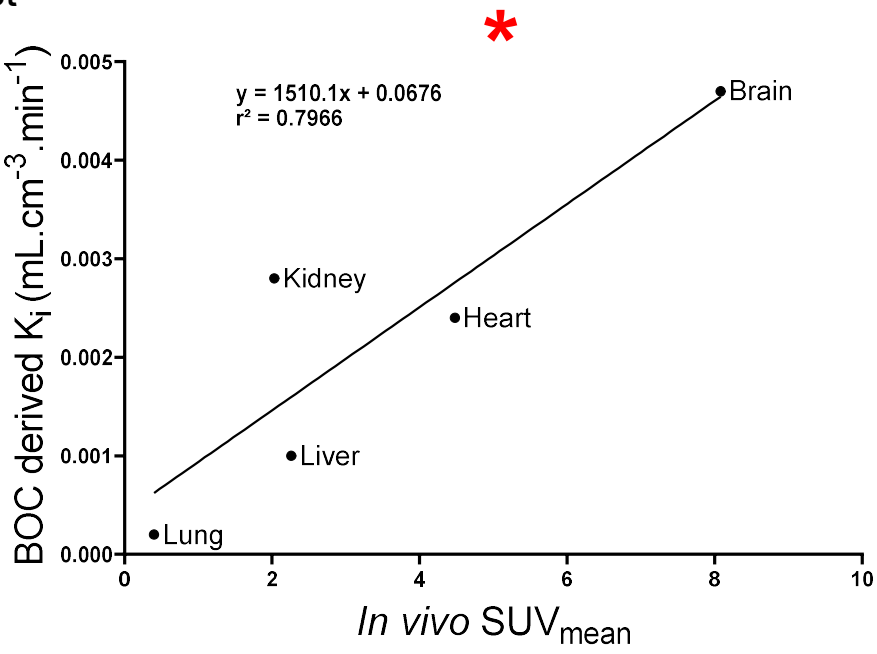


P= 0.0594, Pearson's correlation, n=1



P= 0.0416, Pearson's correlation, n=1

André H. Dias et al. EJNMMI Res. 12: 15. (2022). https://doi.org/10.1186/s13550-022-00884-0

Device allows for quantification of docetaxel and metabolites

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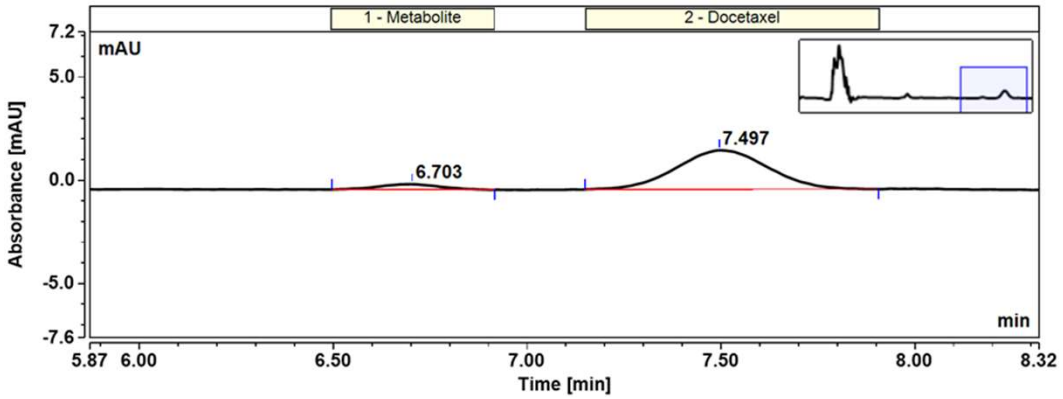

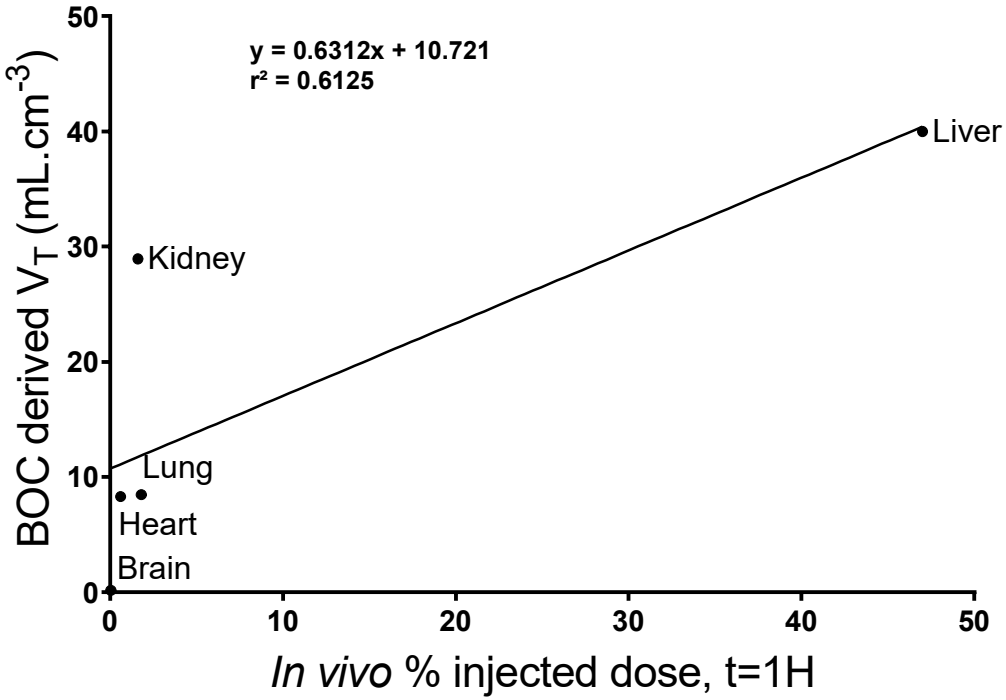

Astrid A. M. van der Veldt *et al.* Journal of Nuclear Medicine and Molecular Imaging. 37(10): p1950–1958; (2010). DOI: https://doi.org/10.1007/s00259-010-1489-y

P= 0.1176, Pearson's correlation, n=1

Conclusions

- The novel device is capable of housing 5 transwell inserts with even flow through all compartments
- Transwell dual seeding method allows for fluid separation of all compartments without the need for a common medium
- The device can be used to assess rate of influx into tissue, with potential for more accurate predictions of kinetic parameters upon further development
- The device allows for the detection of metabolites as well as assessment of their distribution
- There is clear bias in the elimination compartments (kidney/liver)

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Future work

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- Slowly increase complexity of the organ compartments
- Incorporate oral absorption via intestinal compartment

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Supervisors:

Dr. Adriana Tavares
Dr. Mark MacAskill
Prof. Paddy Hadoke

Edinburgh Imaging
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Edinburgh College of Art

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Richard Collins, ECA
Anne Grant, CRIC

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Scottish Imaging Network: A Platform for Scientific Excellence

Thank you!

HPLC LOQ - docetaxel

The HPLC LOQ refers to the lowest amount of a compound that can be accurately detected **AND** quantified reliably and accurately. This is calculated as follows:

$$\boldsymbol{Limit\ of\ quantification} = \mathbf{10} \times \frac{\boldsymbol{Standard\ deviation\ of\ the\ Y\ intercept}}{\boldsymbol{Slope\ of\ the\ calibration\ curve}}$$

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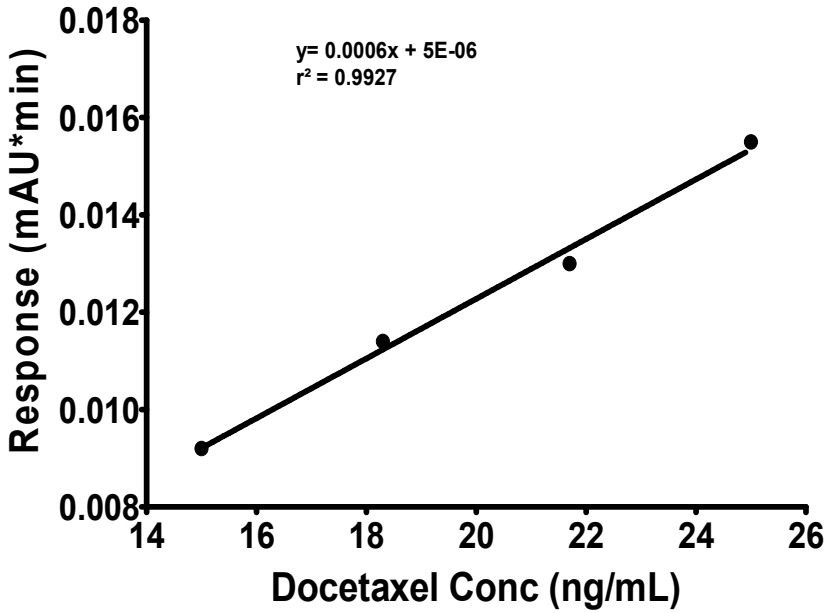
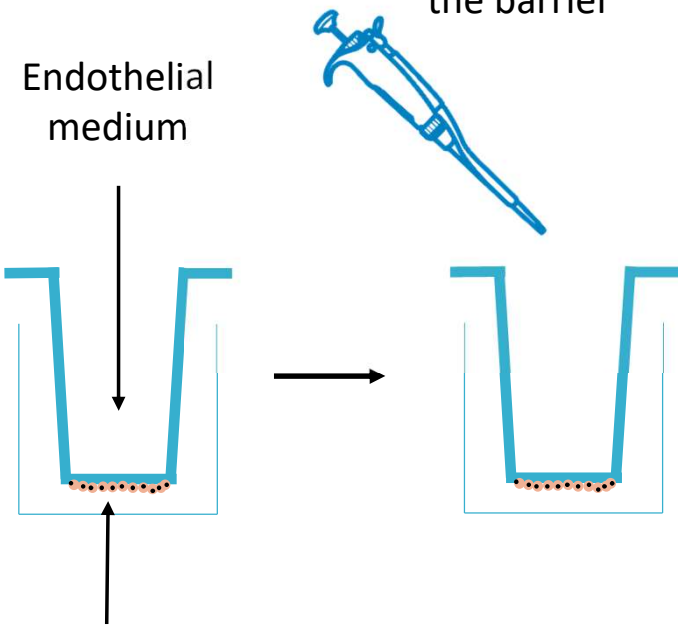


12.3ng/mL

Separation of compartments via endothelial barrier

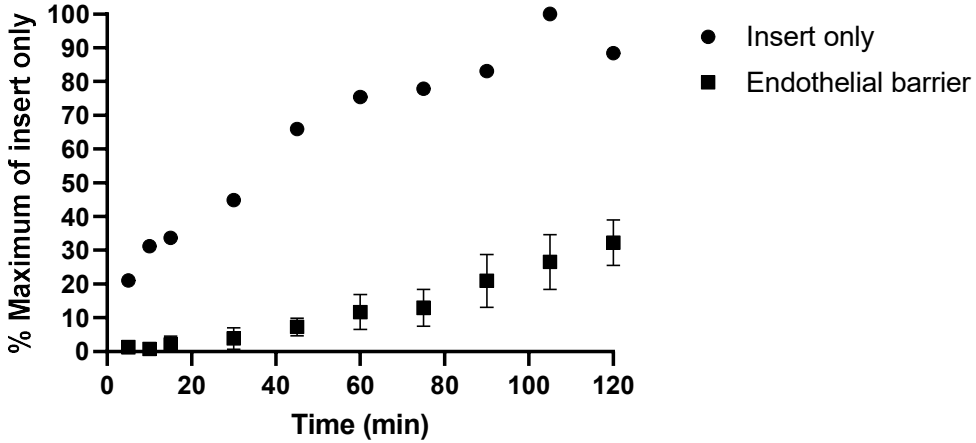
Take small samples over time to measure % Evans blue crossing the barrier

Endothelial medium



0.22mg/mL Evans blue in endothelial medium

Assess using a microplate reader at 610nM

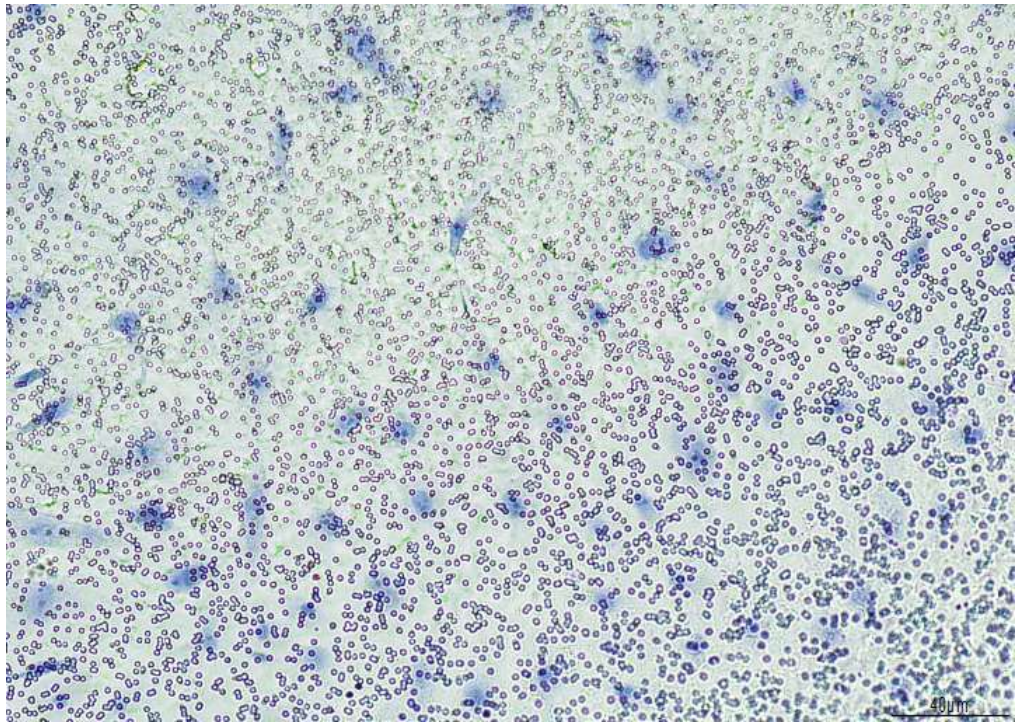


n= 1 insert only, n=3 endothelial barrier (mean ± SEM)

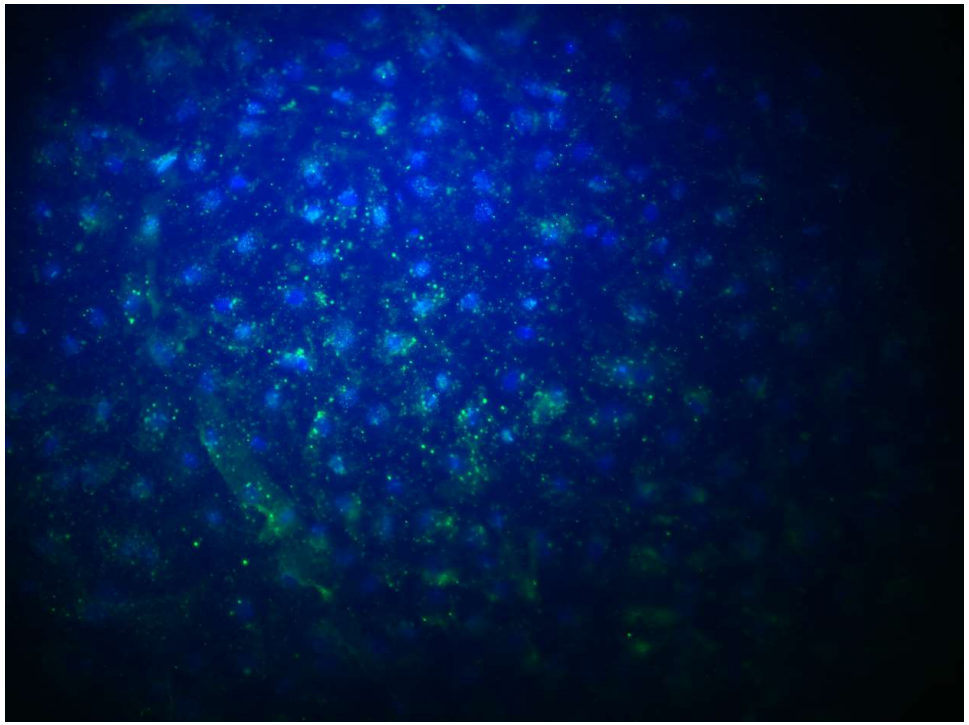
Wu, Meng-Chih et al. *NeuroReport* 32(11): 957-964 (2021). https://doi.org/10.1097/WNR.0000000000001690

Separation of compartments via endothelial barrier

HUVEC nuclei stained with haematoxylin on the underside of a 12-well insert

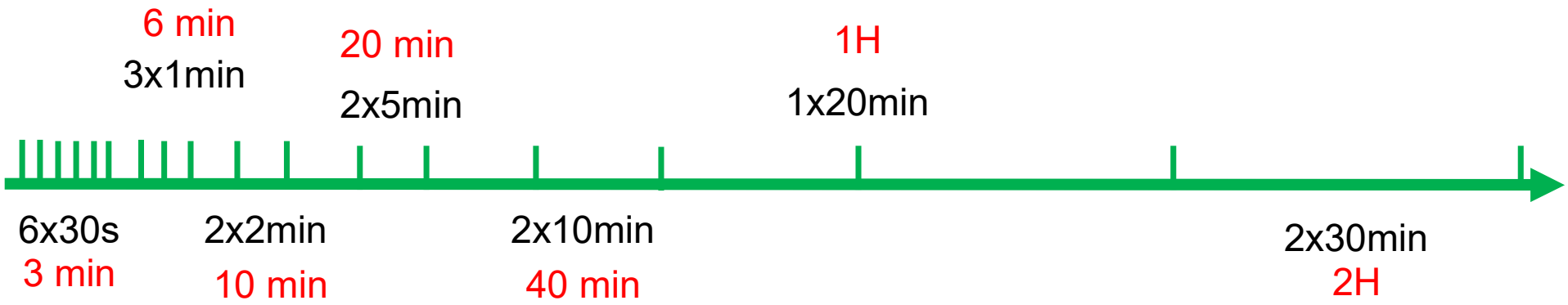


HUVECs stained (badly) with DAPI and CD31 on the underside of a 12-well insert



Kinetic studies

Docetaxel



[^{18}F]FDG