Towards an ADME-Competent 4-Organ-Chip

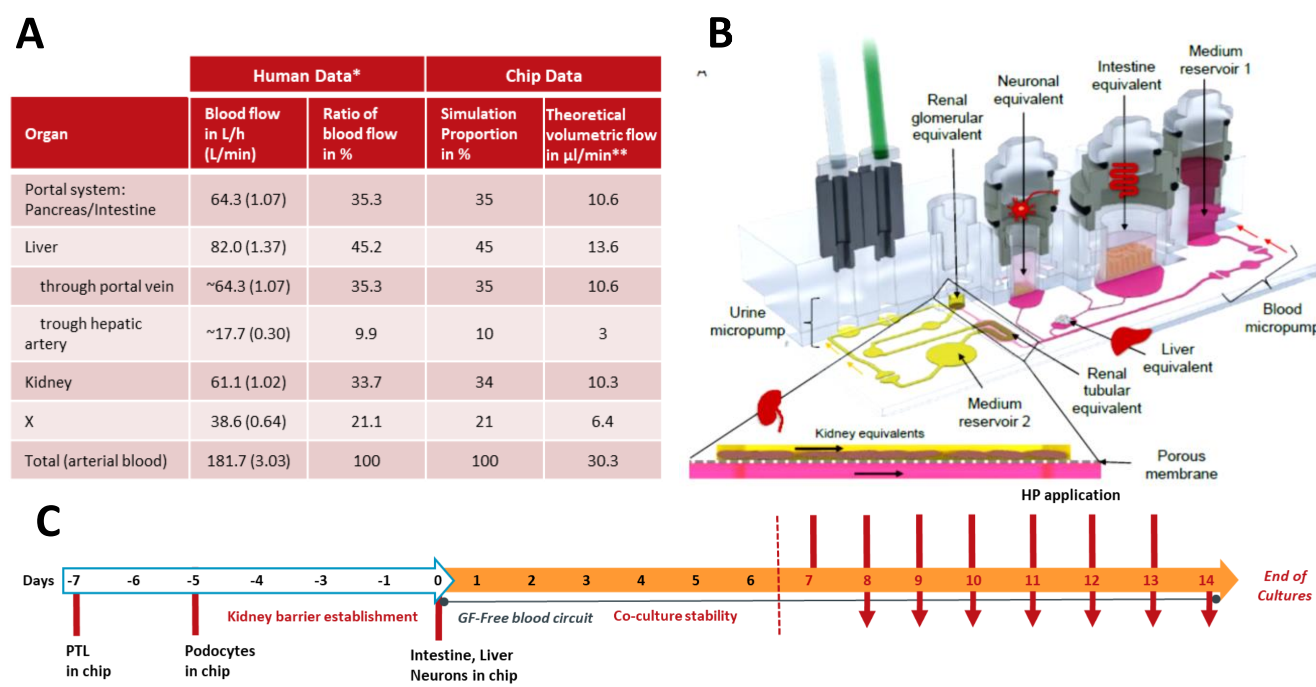
TISSUSE Emulating Human Biology

Beren Ataç[1], Cormac Murphy[2], Anja Wilmes[2], Corinna Magauer[1], Eva Dehne[1], Julianna Kobolák[3], Samantha Bevan[4], Frederic Bois[5], András Dinnyés[3], Paul Walker[4], Paul Jennings[2], Wolfgang Moritz[6], Uwe Marx[1]

[1]TissUse GmbH, Berlin, Germany; [2]VU Amsterdam, Amsterdam, Netherlands; [3]BioTalentum Ltd, Gödöllő, Hungary; [4]Cyprotex Discovery Ltd, Cheshire, United Kingdom; [5]Certara, Sheffield, United Kingdom; [6]InSphero AG, Schlieren, Switzerland.

Abstract

Complex human in vitro ADME models involving co-culture of key organs to mimic certain exposure routes present a challenge to establish physiologically relevant organ models as well as physiologically based pharmacokinetic (PBPK) distribution behavior in the culture environment. In our recent study, we developed a PBPK compliant ADME 4-Organ-Chip (Chip4) with a downscale factor of 1:100.000 of human body. The integration of an intestinal barrier model for absorption, liver microtissues for the main metabolism, a kidney model with proximal tubular-like cells (PTL) and podocytes for excretion, and neuronal spheroids as a potential target organ were optimized in the chip and co-cultured for 14 days. We exposed the Chip4 to Haloperidol (HP), an antipsychotic medication in butyrophenone family with a repeated dose to observe their metabolite induced toxic effects on an organ-specific level. We aim to develop a testing system as a potential new approach methodology for toxicological testing and to increase predictability in the preclinical stage with the multi-organ-chip platform.



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Organ	Human Data*: Blood flow in L/h (L/min)	Human Data*: Ratio of blood flow in %	Chip Data: Simulation Proportion in %	Chip Data: Theoretical volumetric flow in µl/min**
Portal system: Pancreas/Intestine	64.3 (1.07)	35.3	35	10.6
Liver	82.0 (1.37)	45.2	45	13.6
through portal vein	~64.3 (1.07)	35.3	35	10.6
trough hepatic artery	~17.7 (0.30)	9.9	10	3
Kidney	61.1 (1.02)	33.7	34	10.3
X	38.6 (0.64)	21.1	21	6.4
Total (arterial blood)	181.7 (3.03)	100	100	30.3

Figure 1 (A) Simulated organ perfusion rates in humans (*Symcyp) and in Chip4. **(B)** PBPK modelled Chip4 architecture and culture compartments. **(C)** Experimental set up of the ADME-N Chip4 co-culture with 7 day repeated Haloperidol (HP) application in 2 experiments.

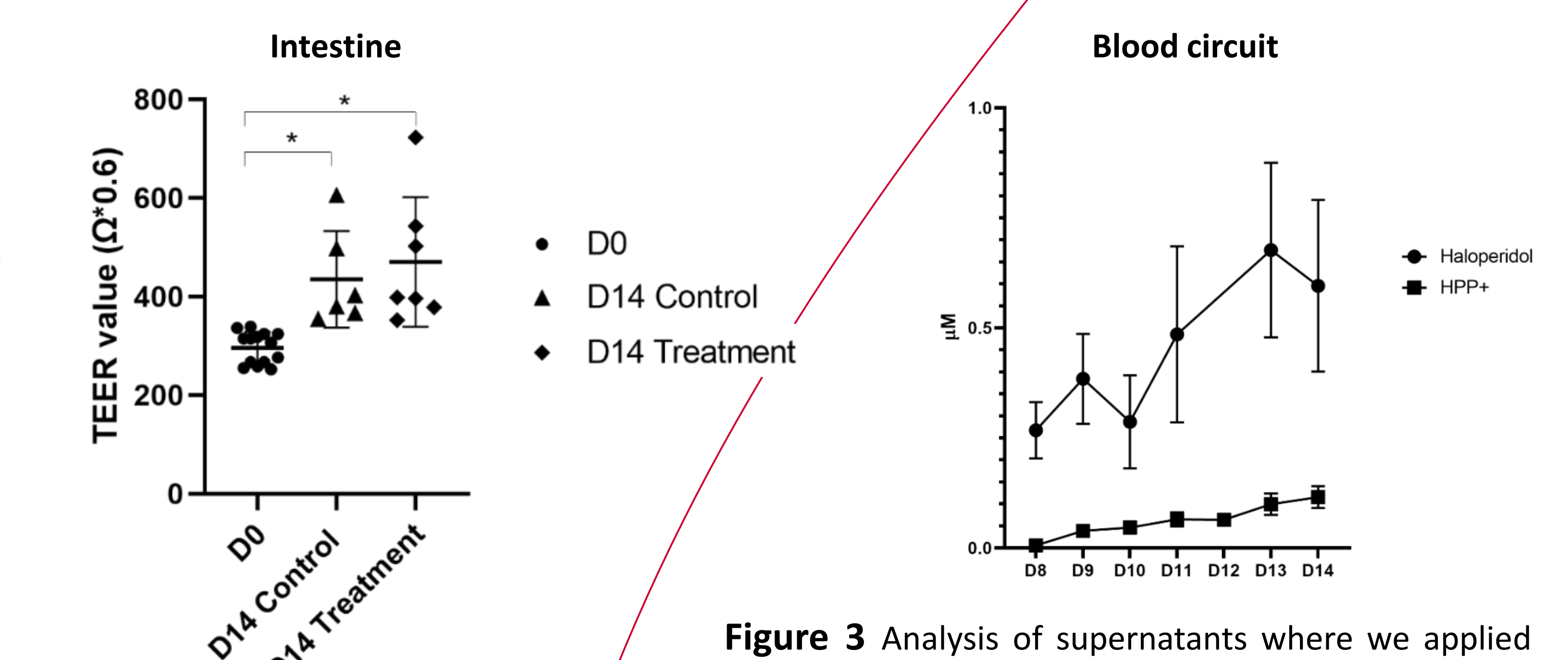

Figure 2 TEER measurement was done in at D0 and D14 of the experiment to test the barrier integrity of intestine.

Figure 3 Analysis of supernatants where we applied 1,5uM HP was performed using ultra-high performance chromatography coupled with triple quadrupole mass spectrometry. Compound concentrations were estimated against a standard curve of the analyte of interest, plotting the concentration vs. analyte/internal standard peak area ratio.

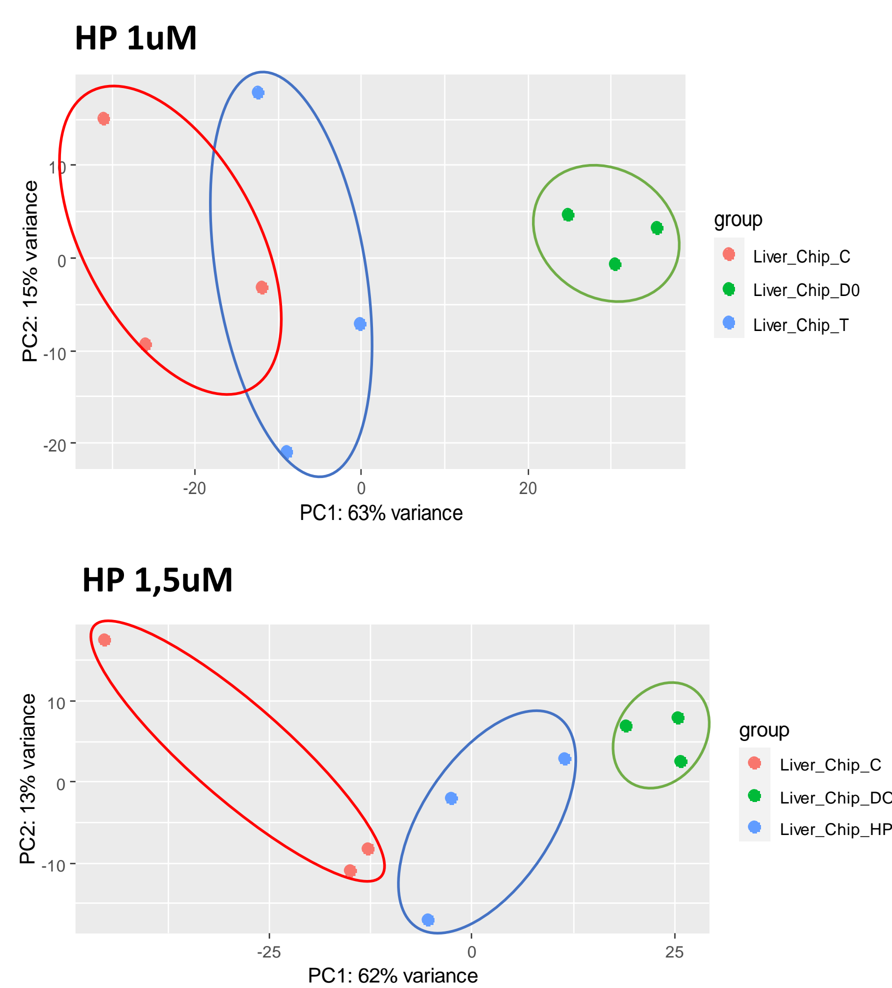

Figure 4 Samples from 2 experiments were analysed for full transcriptome "TempoSeq" (Bioclavis, LTD in Glasgow). Some samples were removed due to low sums of probe read counts (5000-12000). An unsupervised PCA plot of all Day Zero, Day 14 Control and Day 14 HP treated samples from the liver compartment of the chip was made.

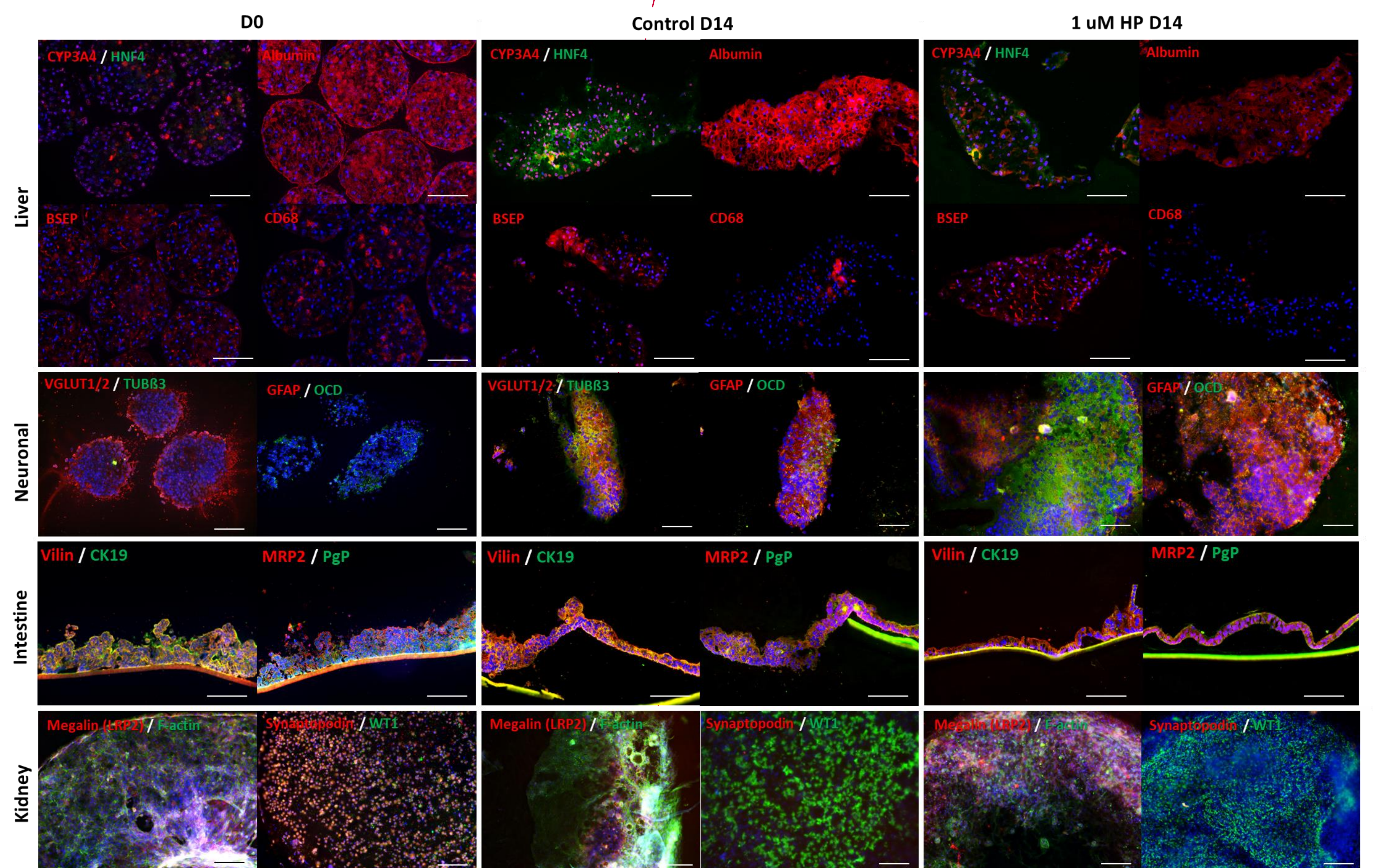

Figure 5 Immunoflourescent analysis of the cryosectioned organ models at day 0 and day 14 where we applied 1uM HP. Kidney: left images PTL, right images Glomerular compartment. Scale bar: 100um

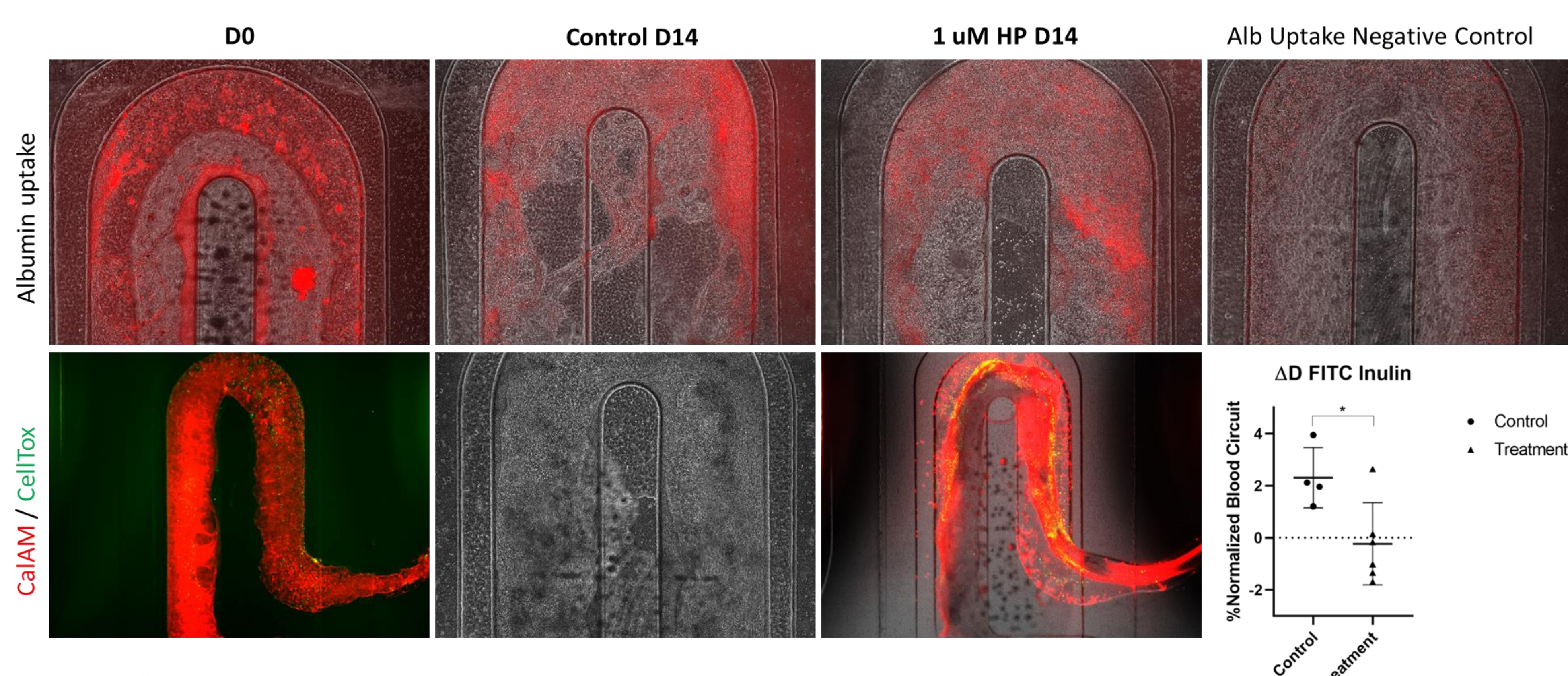

Figure 6 PTL cells were challenged for Albumin uptake at D0 and D14. As negative control we cultured HaCaT cells in the PTL compartment. CalceinAM is used for efflux testing while CellTox stained the dead cells. HP is known to inhibit efflux proteins in proximal tubuli where we see the retained dye in the treatment group. FITC-Inulin is used to test the barrier properties of the kidney circuit where in the treatment we saw a reduction of barrier properties.

Contact

beren.atac@tissuse.com

https://www.tissuse.com/en/

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