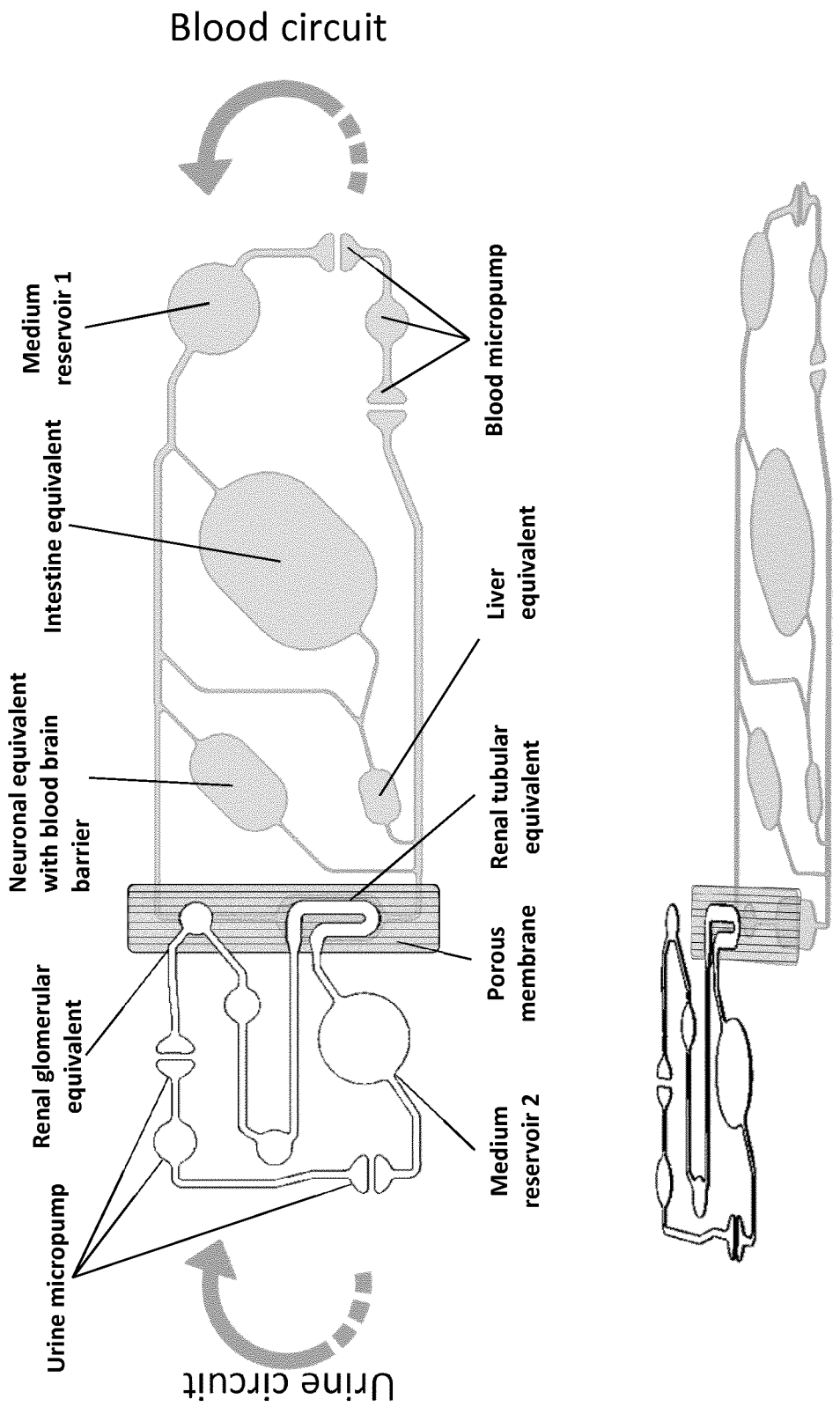


Fig. 1: Exemplary ADME-N footprint (top) and isometrical view on separate layers (bottom) exemplifying the positioning of a membrane between the two circuits.

Fig. 2: Experimental design of iPSC chips and primary tissue chips



Fig. 2A: Experiment 1.1: iPSC Chips – 3 chips

- iPSC liver equivalent
- iPSC intestine equivalent
- iPSC neuronal equivalent with blood brain barrier printed
- RPTECs kidney

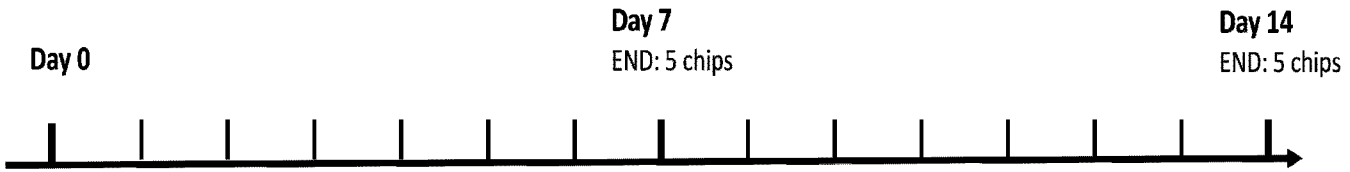


Fig. 2A: Experiment 1.2: Primary tissue Chips – 10 chips

- Primary liver tissue
- Primary small intestine
- iPSC neuronal equivalent with blood brain barrier printed
- RPTECs kidney

Fig. 2 (*cont'd*): Experimental design of iPSC chips and primary tissue chips

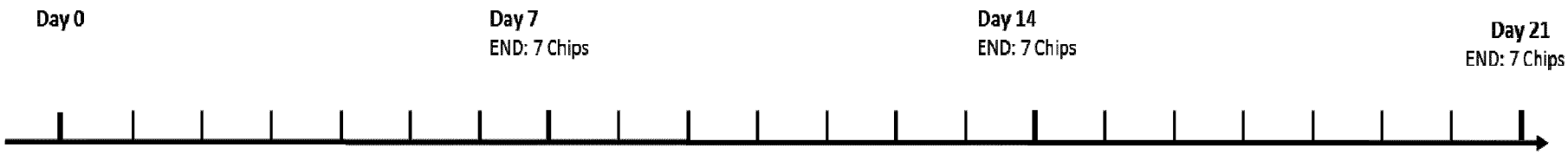


Fig. 2B: Experiment 2: iPSC Chips – 21 chips

- iPSC liver equivalent
- iPSC intestine equivalent
- iPSC neuronal equivalent with blood brain barrier
- iPSC renal equivalent

Fig. 3: Glucose concentration and LDH activity of Experiments 1.1 and 1.2, measured daily in the apical compartment of the intestinal equivalent, in the “blood” medium reservoir, and in the “urine” medium reservoir.

Fig. 3A: Experiment 1.1: iPCS Chips – 3 chips

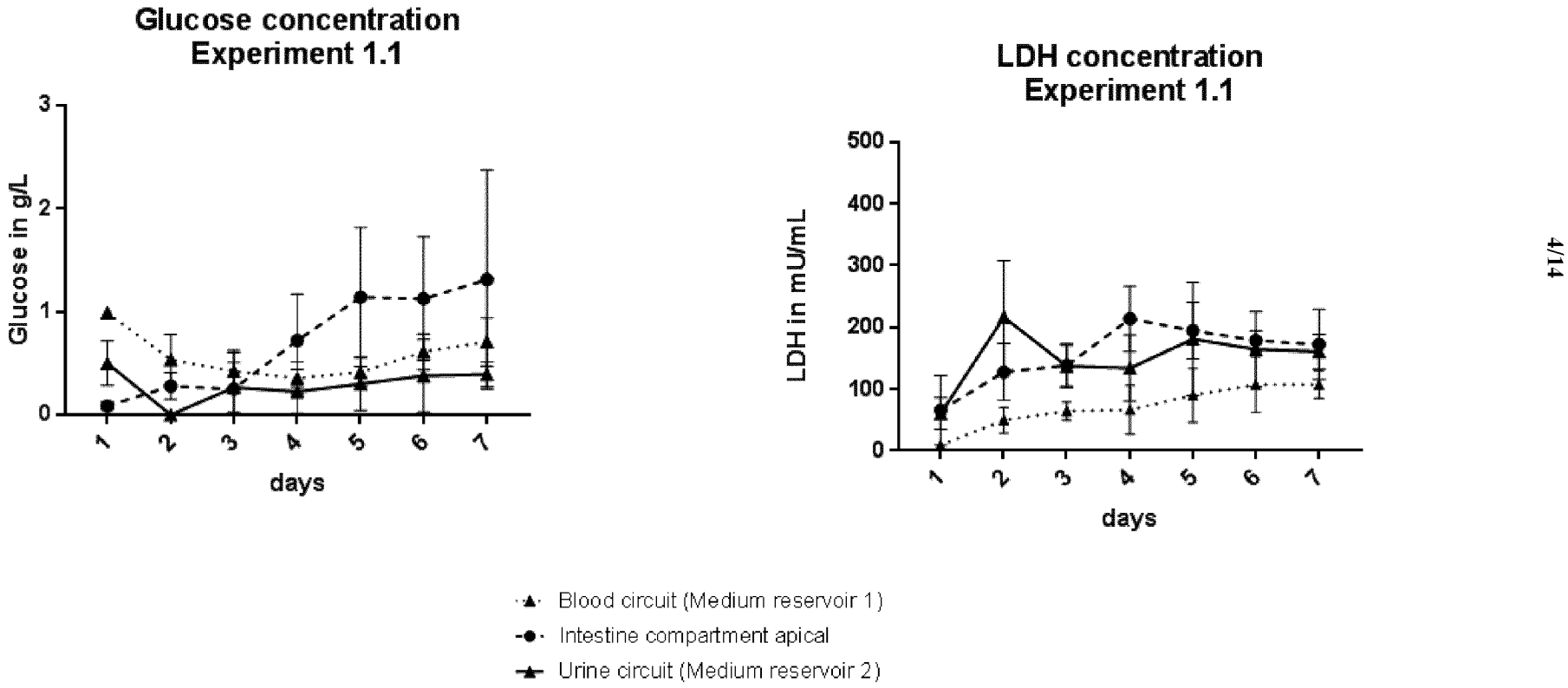


Fig. 3 (*cont'd*): Glucose concentration and LDH activity of Experiments 1.1 and 1.2, measured daily in the apical compartment of the intestinal equivalent, in the "blood" medium reservoir and "urine" medium reservoir.

Fig. 3B: Experiment 1.2: Primary tissue Chips – 10 chips

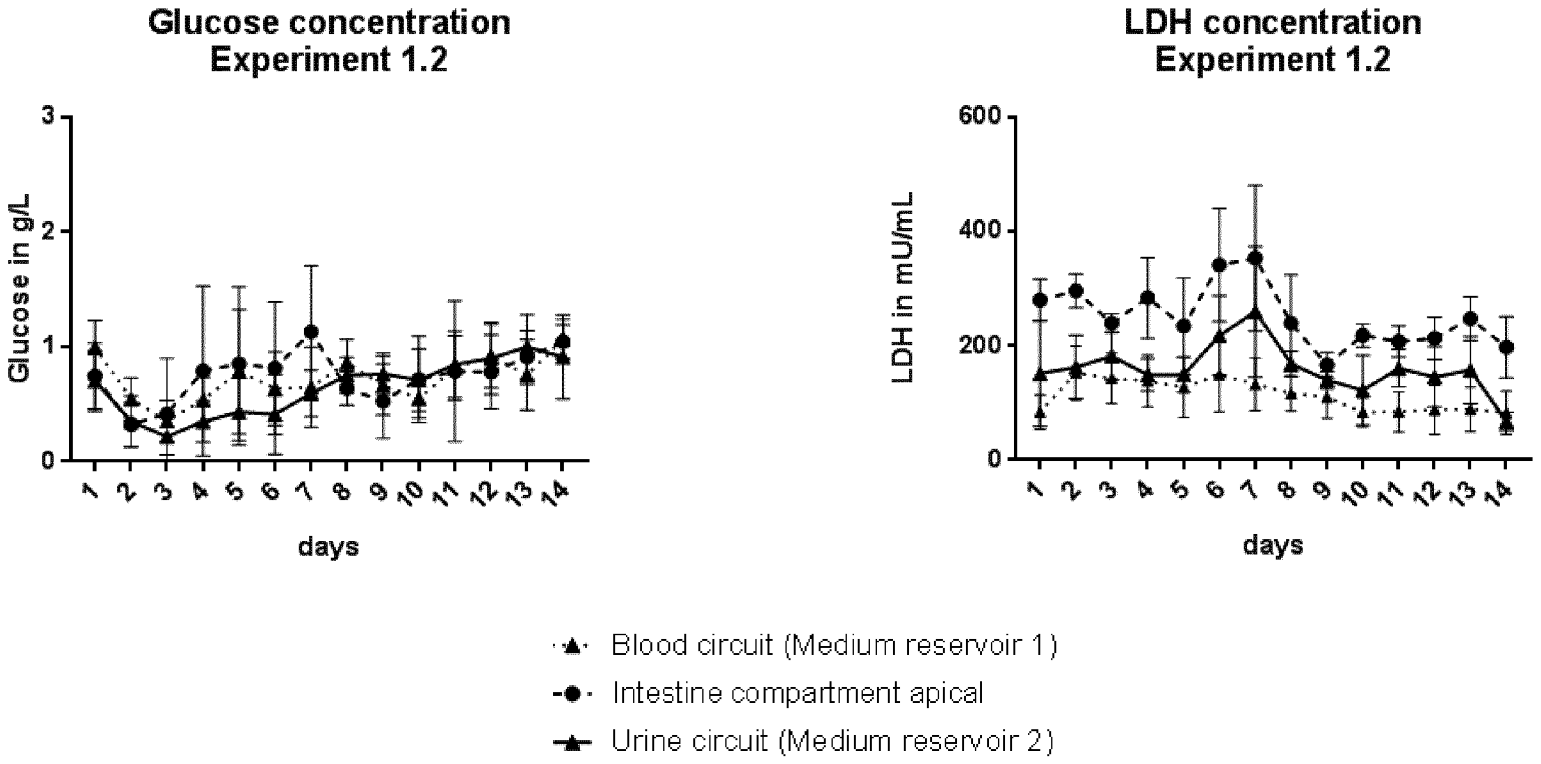
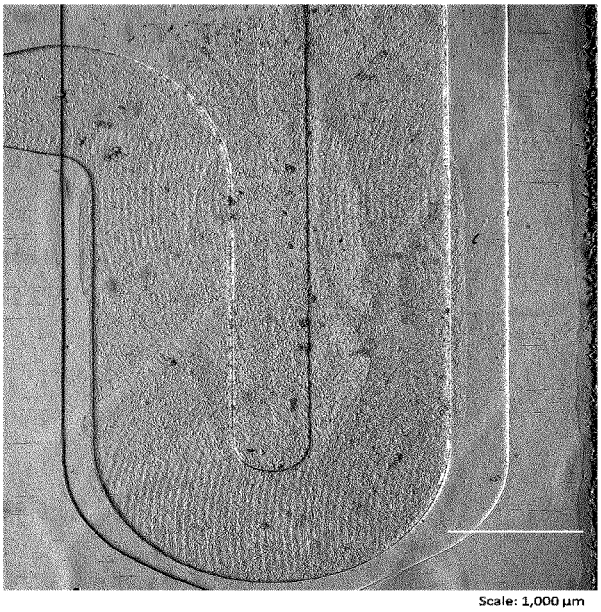
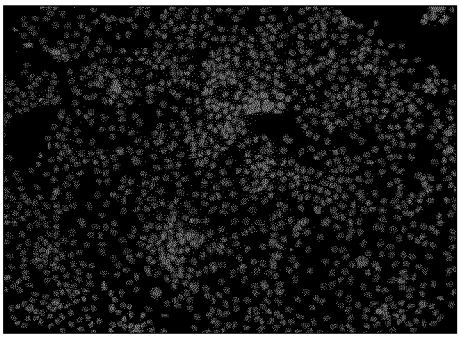


Fig. 4: Renal tubular equivalent fully seeded with human RPTECs (Experiment 1.1, day 7). **A:** bright field (BF) image on day 7 of culture. **B-D:** immunohistolgy stainings of RPTECs on day 14 (Experiment 1.2) **B:** nucleus stained with DAPI; **C:** cell membranes stained with NaK-ATPase; **D:** cytoskeleton stained with cytokeratin 8/18.

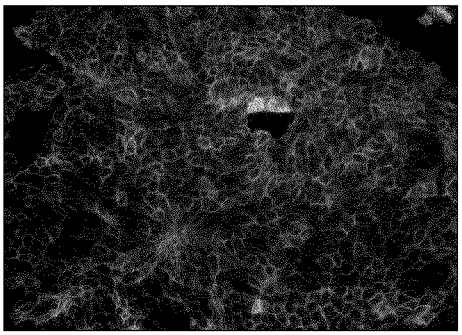
A: BF



B: DAPI



C: NaK-ATPase



D: Cytokeratin 8/18

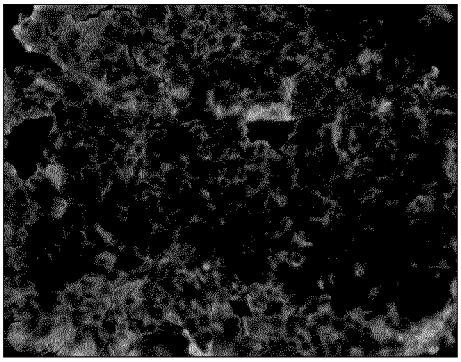


Fig. 5: Aspartate transaminase (AST) concentration measured in the “blood” circuit in medium reservoir 1, and in the “urine” circuit in medium reservoir 2.

Experiment 2: iPSC Chips – 21 chips

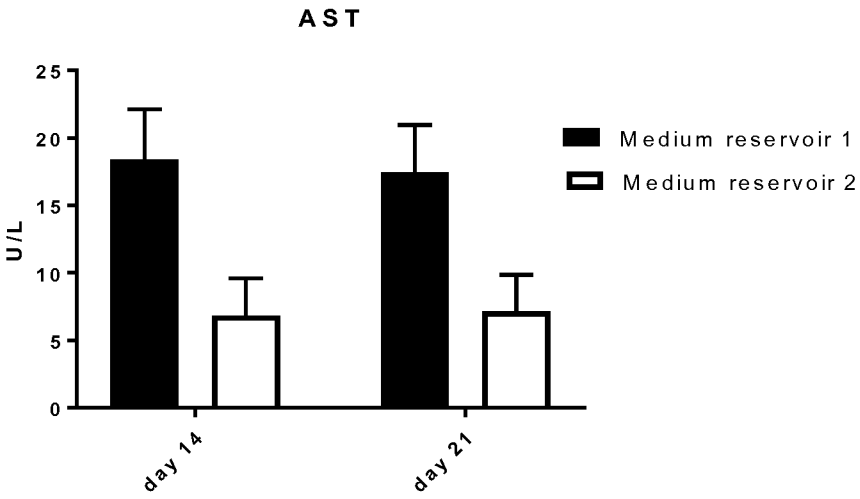
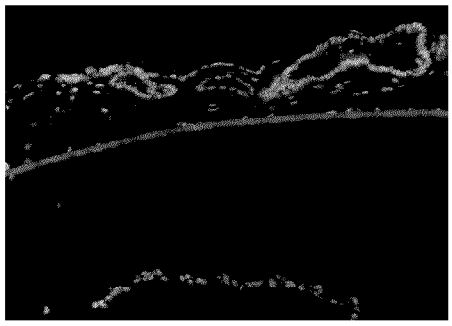


Fig. 6: Exemplary immunohistological examination of an iPSC intestine equivalent with primary endothelial cells on day 7 of ADME-N chip culture (Experiment 1.1). **A:** nucleus stained with DAPI; **B:** primary endothelial cells stained for CD31; **C:** iPSC intestinal organoids stained for NaK-ATPase. **D:** nucleus stained with DAPI; **E:** iPSC intestinal organoids stained for Cyp3A4; **F:** iPSC-derived fibroblasts stained for vimentin.

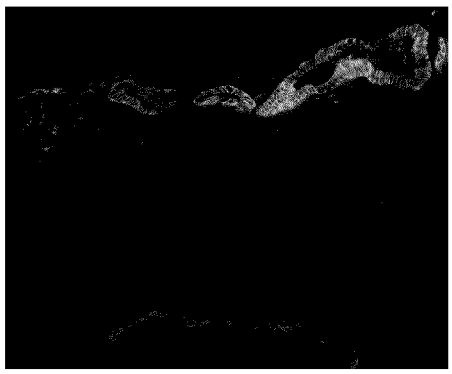
A: DAPI



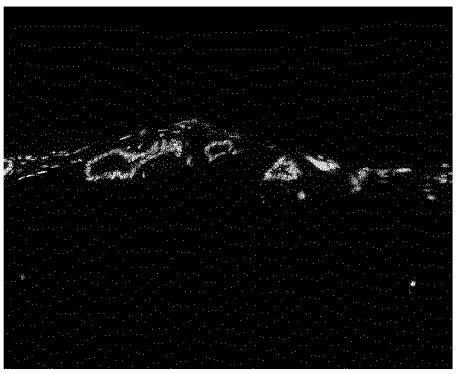
B: CD31



C: NaK-ATPase



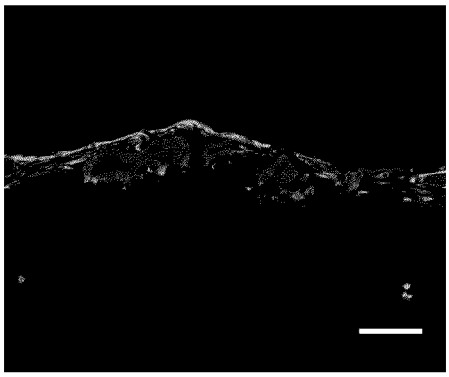
D: DAPI



E: Cyp3A4



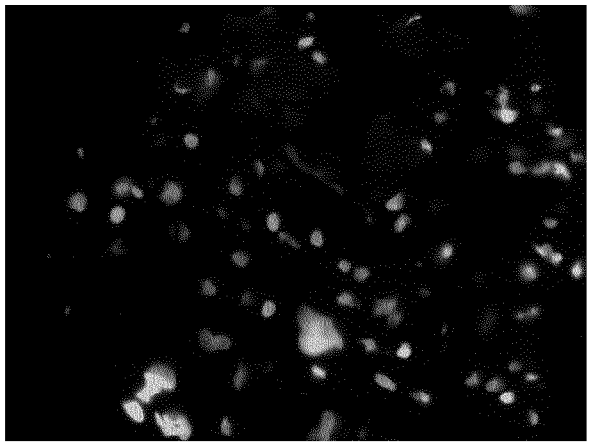
F: Vimentin



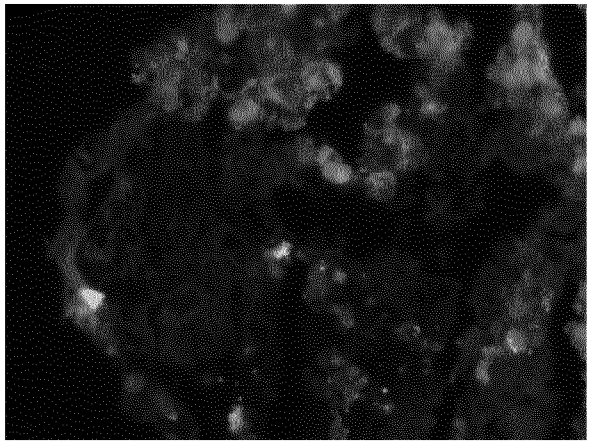
Scale: 100µm

Fig. 7: Exemplary immunohistological examination of primary liver equivalents (InSphero) cultivated for two weeks in the ADME-N chip (Experiment 1.2). **A:** nucleus stained with DAPI; **B:** albumin; **C:** Cyp3A4.

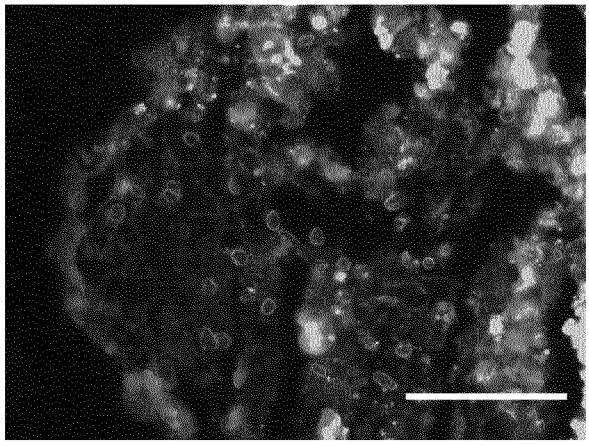
A: DAPI



B: Albumin



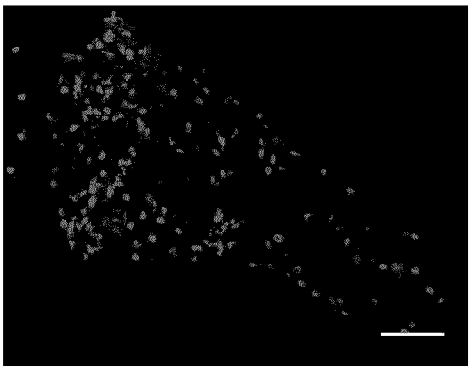
C: Cyp3A4



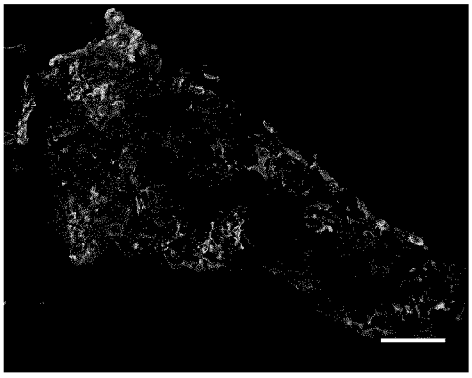
Scale: 100µm

Fig. 8: Exemplary immunohistological examination of iPSC-derived liver equivalent cultivated for three weeks in the ADME-N chip (Experiment 2). **A:** nucleus stained with DAPI; **B:** iPSC fibroblasts stained for vimentin; **C:** cytoskeleton of hepatocytes stained with cytokeratin 8 /18, **D:** nucleus stained with DAPI; **E:** tight junctions stained with ZO-1; **F:** albumin.

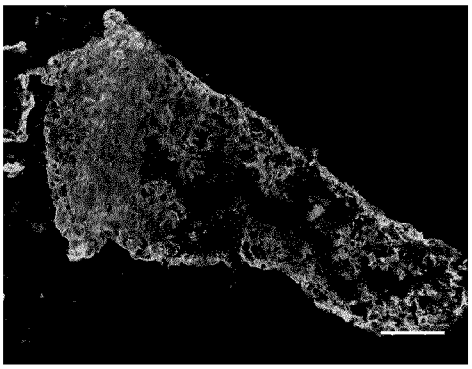
A: DAPI



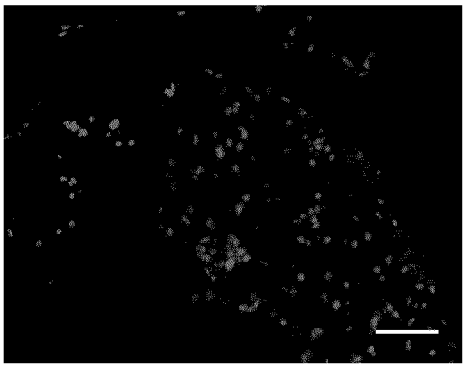
B: Vimentin



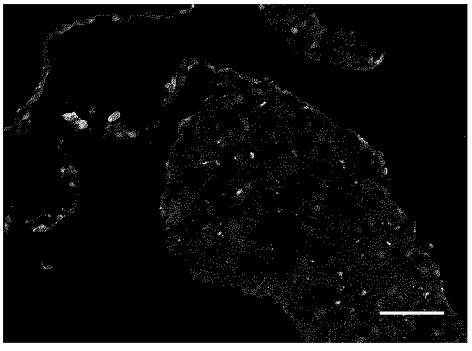
C: Cytokeratin 8 /1



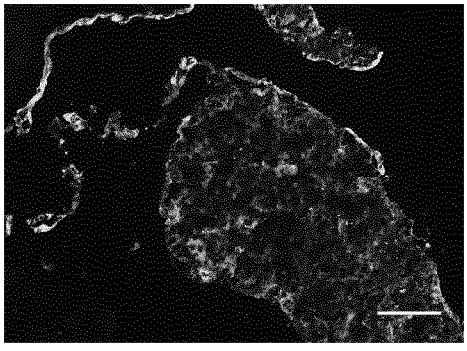
D: DAPI



E: ZO-1



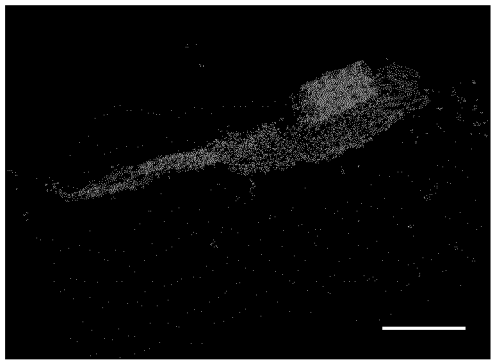
F: albumin



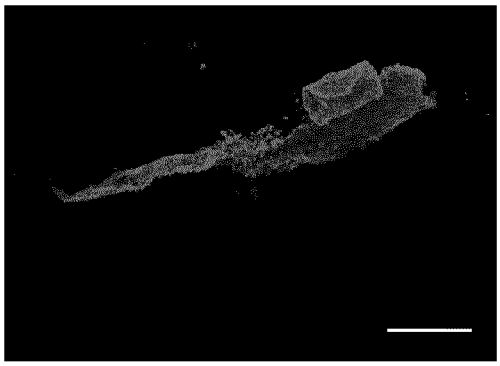
Scale: 100µm

Fig. 9: Exemplary immunohistological examination of iPSC neuronal equivalents cultivated for two weeks in the ADME-N chip (Experiment 1.2). **A:** nucleus stained with DAPI; **B:** neuronal cells stained with b Tubulin III; **C:** primary endothelial cell stained with vWF (Von-Willebrand-Faktor); **D:** bright field image of neuronal spheroids lying on printed hydrogel including endothelial cells.

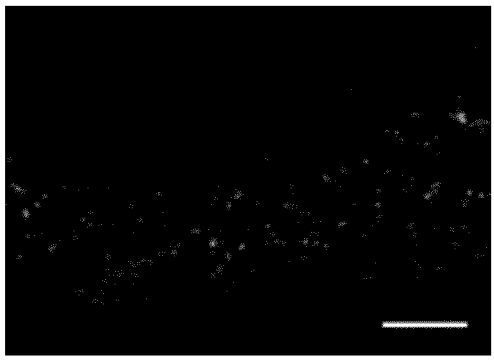
A: DAPI



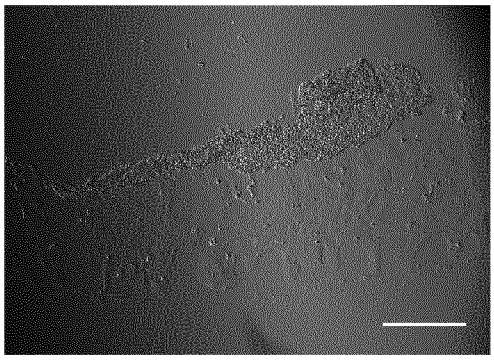
B: B Tubulin III



C: vWF



D: BF



Scale: 250µm

Fig. 10: LDH concentration measured in the apical compartment of the intestinal equivalent, in the blood circuit in medium reservoir 1, and in the urine circuit in medium reservoir 2. For Experiment 2. The barrier function of the intestine equivalent and the renal equivalent is shown by different concentrations of LDH in all three compartments.

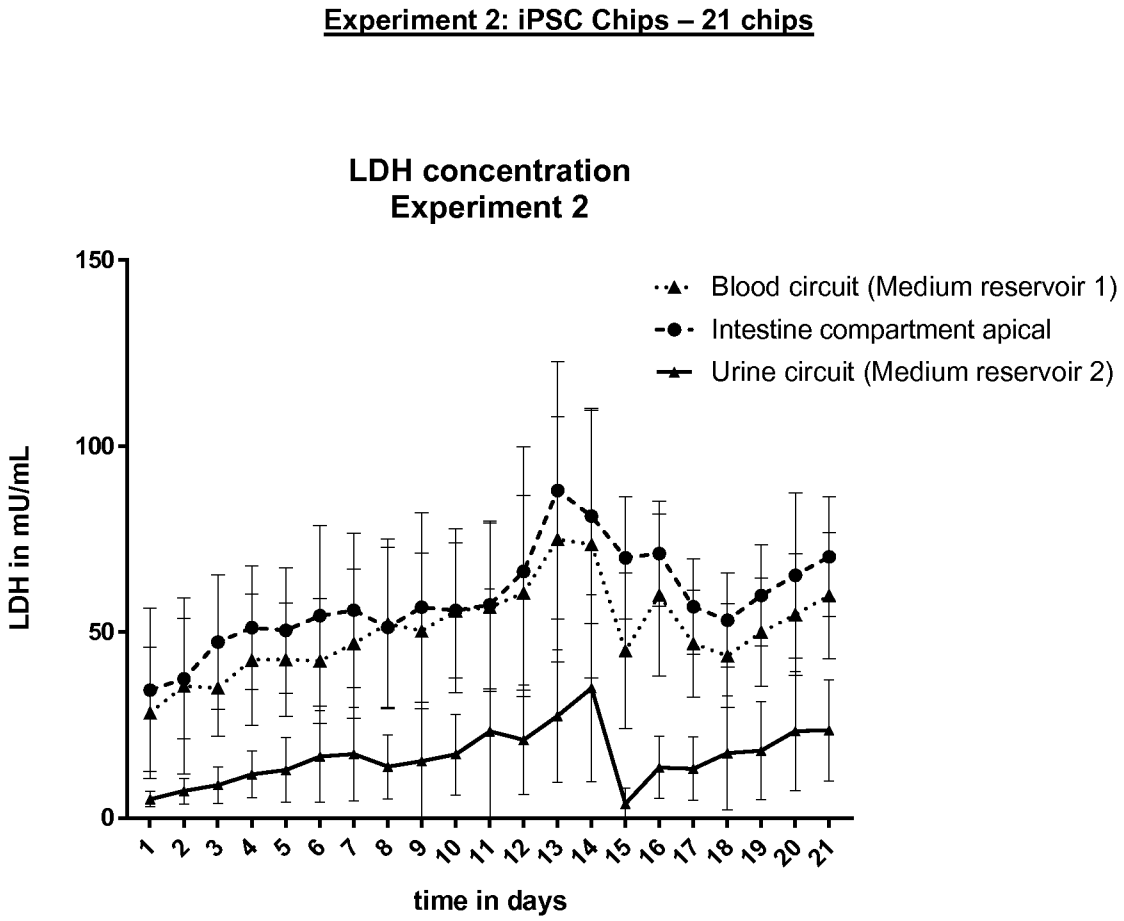
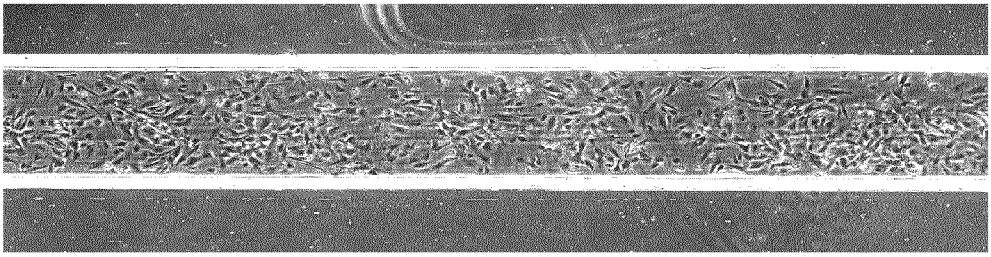
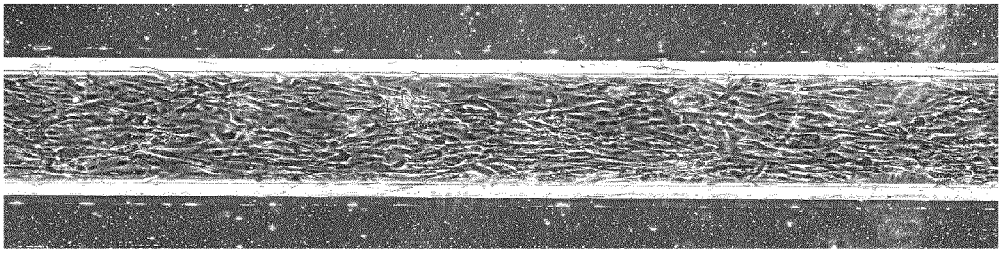


Fig. 11: iPSC derived endothelial cells elongate in the channels of the multi-organ-chip. High expression of the endothelail marker CD31 on day 8 of chip culture.

Day 0



Day 8



Day 8:

DAPI **CD31 8 (Scale 100µm)**

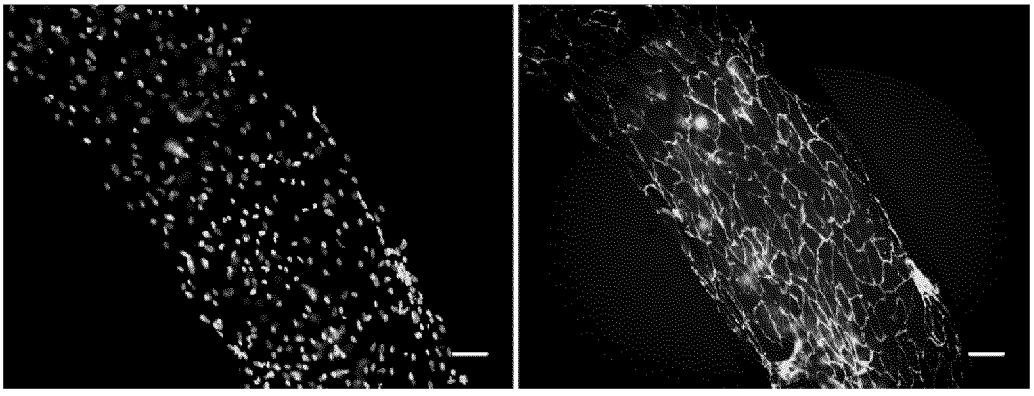


Fig. 12: Partial cross-sectional schematic view of ADME-Chip. A = Administration, D = Distribution, M = Metabolism, E = Excretion.

