TISSUSE Emulating Human Biology

TOWARDS A PERSONALIZED KIDNEY-ON-A-CHIP DERIVED FROM PLURIPOTENT STEM CELLS

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INTRODUCTION

Our understanding of the mechanisms involved in the development and progression of renal disease is limited due to the lack of functional *in vitro* models that can accurately emulate human physiological processes. While microphysiological systems enable the co-cultivation of multiple cell types under nearly physiological conditions, these systems are typically equipped with heterogenous cell populations sourced from multiple donors with diverse genetic backgrounds, which limits their applications in personalized medicine. Here, we generate an autologous kidney-on-a-chip that encompasses human iPS cell-derived podocytes and proximal tubule epithelial cells from a single donor. The renal cells are seeded into the HUMIMIC Chip4, which enables the long-term co-cultivation of the renal model with up to three additional autologous organ equivalents with a defined fluid flow and shear stress. The final maturation of the iPS cell-derived podocytes and tubular cells occurs within the multi-organ-chip, thereby allowing the cells to form a barrier that enables glomerular filtration and tubular reabsorption. After the renal cells' final maturation, the co-culture can be maintained for at least 14 days.

RESULTS

HUMIMIC CHIP4 CULTURE

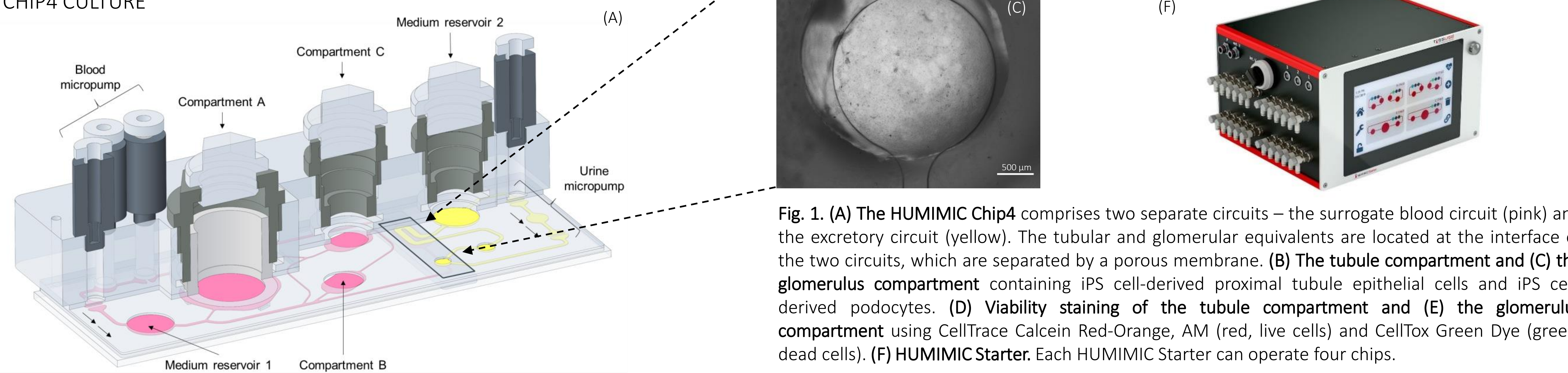

Fig. 1. (A) The HUMIMIC Chip4 comprises two separate circuits – the surrogate blood circuit (pink) and the excretory circuit (yellow). The tubular and glomerular equivalents are located at the interface of the two circuits, which are separated by a porous membrane. **(B) The tubule compartment and (C) the glomerulus compartment** containing iPS cell-derived proximal tubule epithelial cells and iPS cell-derived podocytes. **(D) Viability staining of the tubule compartment and (E) the glomerulus compartment** using CellTrace Calcein Red-Orange, AM (red, live cells) and CellTox Green Dye (green, dead cells). **(F) HUMIMIC Starter.** Each HUMIMIC Starter can operate four chips.

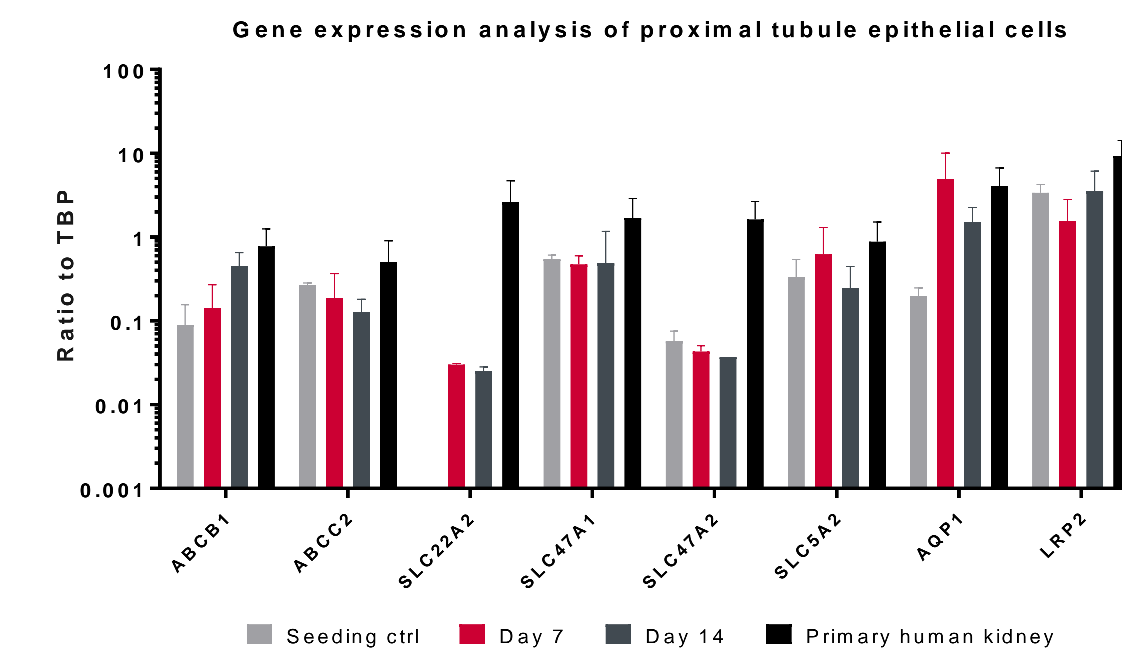

Fig. 2. Gene expression analysis of samples collected from the tubule compartment of the HUMIMIC Chip4. Mean expression values ± s.d. of selected tubular markers were analyzed by RT-qPCR for samples collected on day -4 (seeding control, n=3 biological replicates), day 7 (n=8 biological replicates) and day 14 (n=4 biological replicates) of the chip culture. RNA from primary human adult kidney (n=3 biological replicates) served as a positive control. The data was normalized to the housekeeping gene TBP (TATA-box binding protein). Tubular markers included ABCB1 (multidrug resistance protein 1, MDR1), ABCC2 (multidrug resistance protein 2, MRP2), SLC22A2 (organic cation transporter 2, OCT2), SLC47A1 (multidrug and toxin extrusion 1, MATE1), SLC47A2 (multidrug and toxin extrusion 2k, MATE2k), SLC5A2 (sodium glucose transporter 2, SGLT2), AQP1 (aquaporin 1) and LRP2 (megalin).

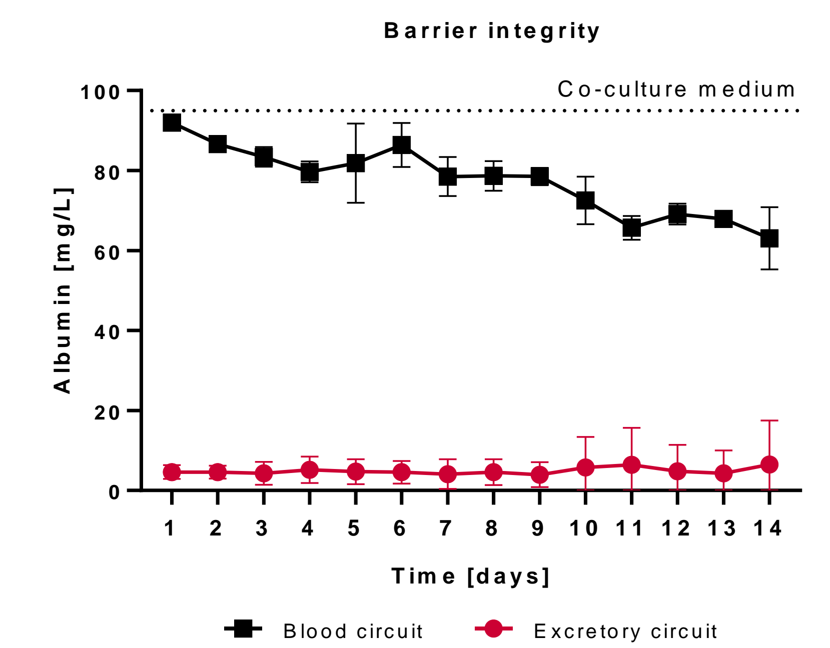

Fig. 3. Kidney-on-a-chip barrier integrity assay. Human albumin concentration in medium samples collected from the blood circuit (black) and the excretory circuit (red) during a 14 day chip culture. Medium samples were collected during the daily medium exchange. Mean values ± s.d. for n=3 biological replicates. The dotted line represents the albumin concentration of the co-culture medium used in the blood circuit. The medium employed in the excretory circuit does not contain human albumin.

iPS CELL-DERIVED RENAL MODELS

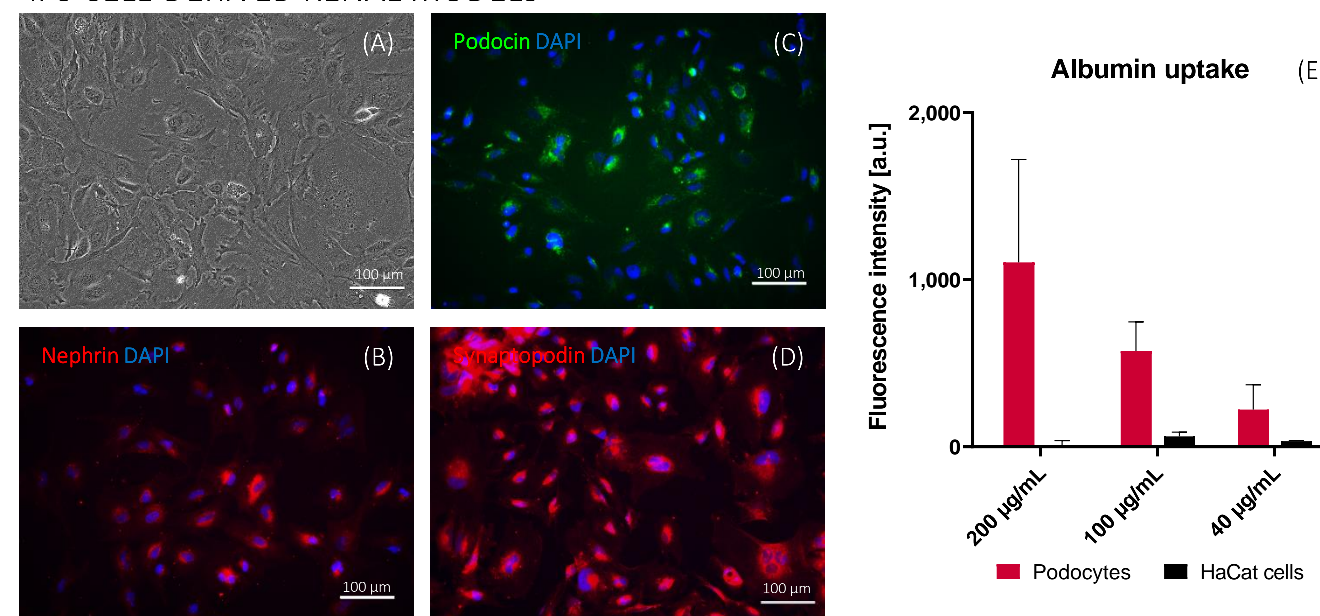

Fig. 4. iPS cell-derived podocytes. (A) Podocyte morphology. The cells exhibit typical podocyte morphology with large, arborized cell bodies, prominent nuclei and foot-like processes. **(B-D) Immunofluorescence staining.** Nephrin, podocin and synaptopodin staining with DAPI counterstaining. **(E) Albumin uptake assay.** Mean fluorescence intensity values ± s.d. of lysed podocytes and HaCat cells (negative control) after incubation with 200/100/40 µg/mL FITC-albumin at 37°C normalized to the FITC-albumin uptake at 4°C with n=3 biological replicates.

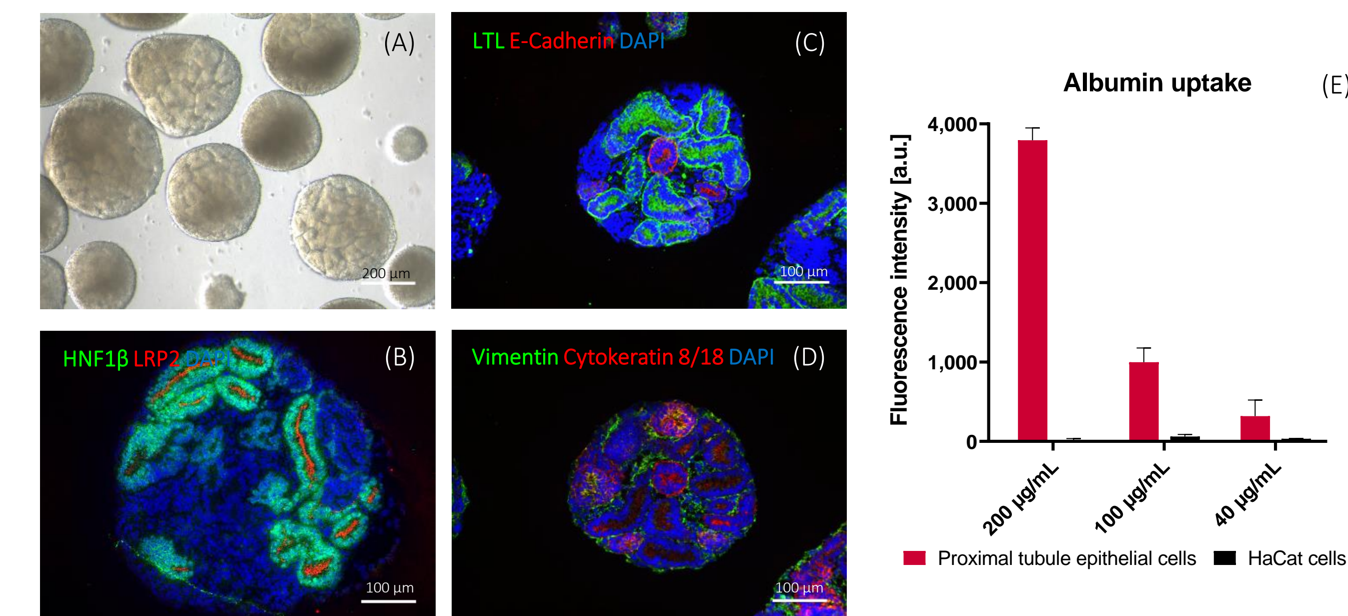

Fig. 5. iPS cell-derived proximal tubule epithelial cells. (A) Tubular organoid morphology. The tubular organoids are round and compact with characteristic tubular structures. **(B-D) Immunofluorescence staining.** HNF1ß/LRP2, LTL/E-Cadherin and Vimentin/Cytokeratin 8/18 staining with DAPI counterstaining. **(E) Albumin uptake assay.** Mean fluorescence intensity values ± s.d. of lysed proximal tubule epithelial cells and HaCat cells (negative control) after incubation with 200/100/40 µg/mL FITC-albumin at 37°C normalized to the FITC-albumin uptake at 4°C with n=3 biological replicates.

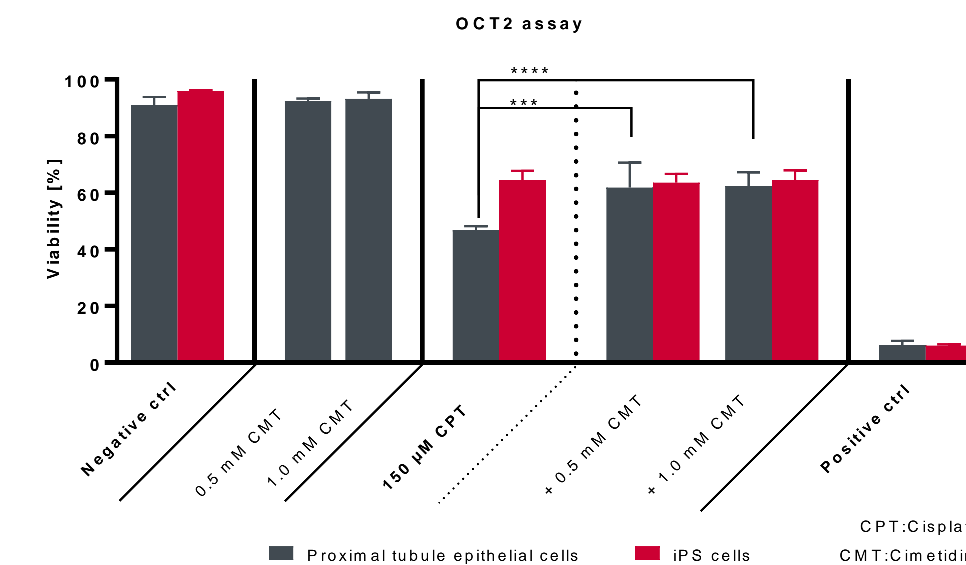

Fig. 6. iPS cell-derived proximal tubule epithelial cells. OCT2 assay. Proximal tubule epithelial cells and iPS cells (control) were treated with the chemotherapeutic agent cisplatin, which is transported into renal cells by the organic cation transporter 2 (OCT2). Cimetidine specifically inhibits OCT2 and can thereby prevent cisplatin-induced nephrotoxicity. Negative control: untreated. Positive control: incubation with 0.1% Triton X-100 for 30 min. Mean viability values ± s.d. for n=3 biological replicates. Differences to the control were evaluated by one-way ANOVA using Dunnett's multiple comparisons post-hoc test, ***$p < 0.001$, ****$p < 0.0001$.

SUMMARY AND OUTLOOK

The developed kidney-on-a-chip constitutes a potent tool for advanced *in vitro* drug trials. The iPS cell-derived renal cells form a barrier that prevents albumin from entering the excretory circuit and they demonstrate a steady expression of key podocyte and tubular markers. Functionality assays further demonstrate the *in vivo*-like characteristic behavior of the employed cells. The kidney-on-a-chip can be used for mechanistic studies of renal development or disease as well as for elaborate safety and efficacy studies.

The combination of the renal model with other organ equivalents enables systemic studies, including ADME experiments. When the employed organ models are differentiated from iPS cells derived from a single patient, the HUMIMIC Chip4 represents a personalized multi-organ-chip. This patient-on-a-chip can be used for the development of personalized therapies. Therefore, the developed autologous kidney-on-a-chip could not only advance future studies of renal disease mechanisms, but it could also pave the way towards personalized medicine.

Taken all together, the developed kidney-on-a-chip has the potential to generate high-quality *in vitro* data predictive of renal drug clearance, reabsorption and nephrotoxicity in humans.

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