KIDNEY-ON-A-CHIP – INTEGRATING GLOMERULAR FILTRATION AND TUBULAR REABSORPTION MODELS

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INTRODUCTION

The kidney plays a crucial role in drug development, as it dictates drug clearance and is a target for drug-induced toxicity. Current *in vivo* and *in vitro* models often fail to predict nephrotoxic effects of candidate drugs in humans, which leads to high attrition rates during drug development. Therefore, an accurate kidney model for multi-organ-chip applications could revolutionize drug trials by providing a relevant *in vitro* platform.

Here, we present a human autologous kidney-on-a-chip that encompasses a glomerular and a tubular model. Induced pluripotent stem (iPS) cell-derived podocytes and proximal tubule epithelial cells are generated and seeded into the HUMIMIC Chip4, which can accommodate up to three additional organ equivalents. After the renal cells' final maturation within the chip, the co-culture can be maintained for at least 14 days.

RESULTS

HUMIMIC CHIP4 CULTURE

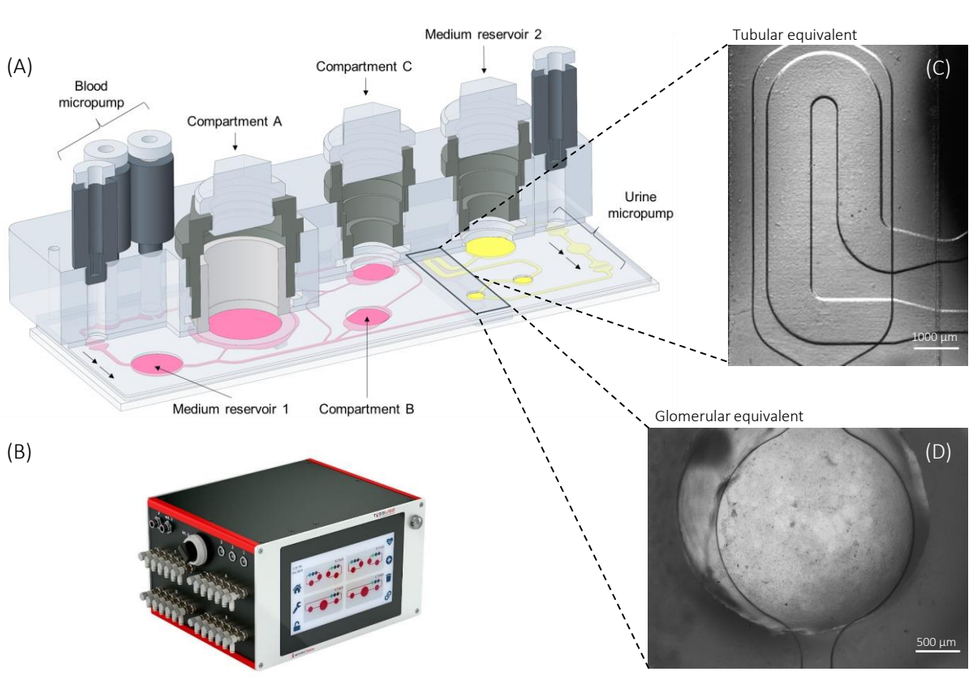

Fig. 1. (A) HUMIMIC Chip4 and (B) HUMIMIC Starter. (A) The HUMIMIC Chip4 comprises two separate circuits – the surrogate blood circuit (pink) and the excretory circuit (yellow). The tubular and glomerular equivalents are located at the interface of the two circuits, which are separated by a porous membrane. (B) Each HUMIMIC Starter can operate four chips. **(C) The tubule compartment and (D) the glomerulus compartment.** (C) iPS cell-derived proximal tubule epithelial cells within the tubular compartment. (D) iPS cell-derived podocytes within the glomerular compartment.

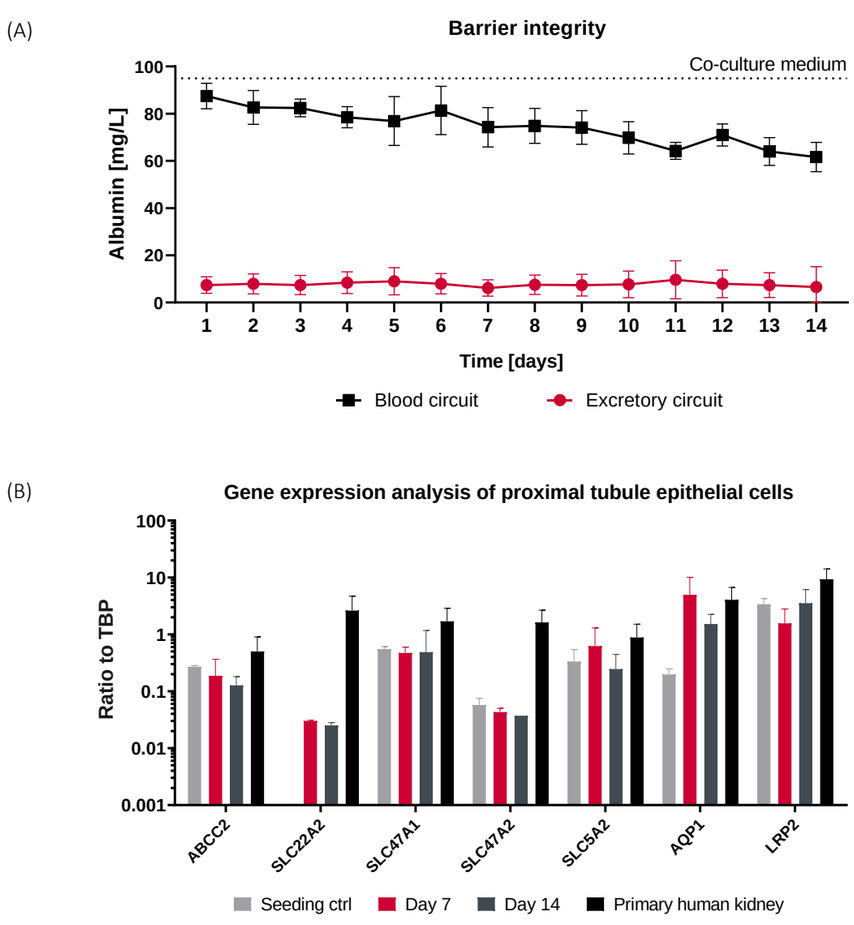

Fig. 2. Kidney-on-a-chip analyses. (A) Barrier integrity assay. Albumin concentration in samples collected from the blood (black) and excretory circuit (red). Mean values ± s.d. for n=6 biological replicates. The dotted line represents the albumin concentration of the co-culture medium used in the blood circuit. The medium employed in the excretory circuit does not contain human albumin. **(B) 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.

IPS-CELL DERIVED RENAL MODELS

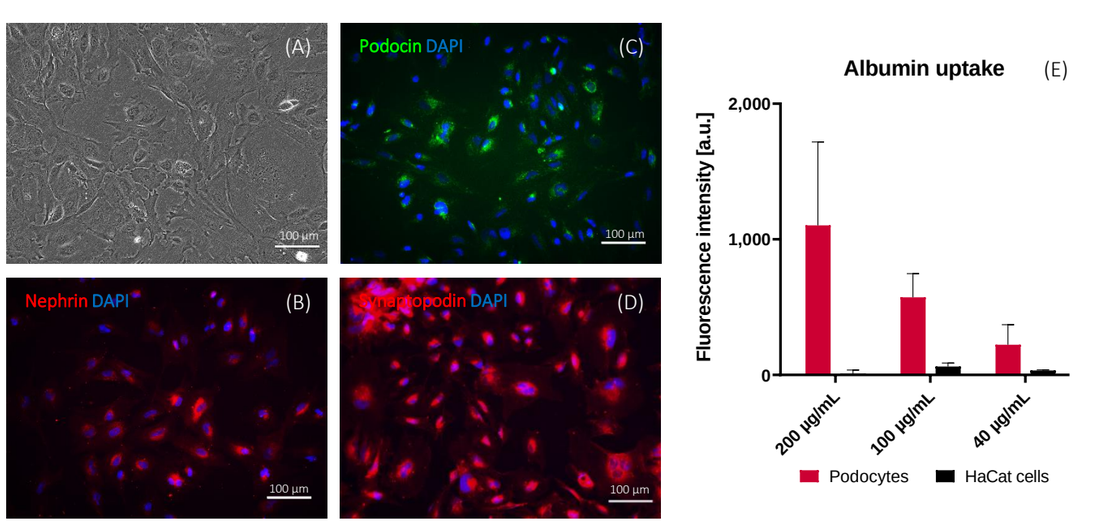

Fig. 3. iPS cell-derived podocytes. (A) Podocyte morphology. The cells exhibit typical podocyte morphology with large, arborized cell bodies, prominent nuclei and foot-like processes. **(B-D) Immunofluorescence staining.** Nephrin, podocin and synaptopodin staining with DAPI counterstaining. **(E) Albumin 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.

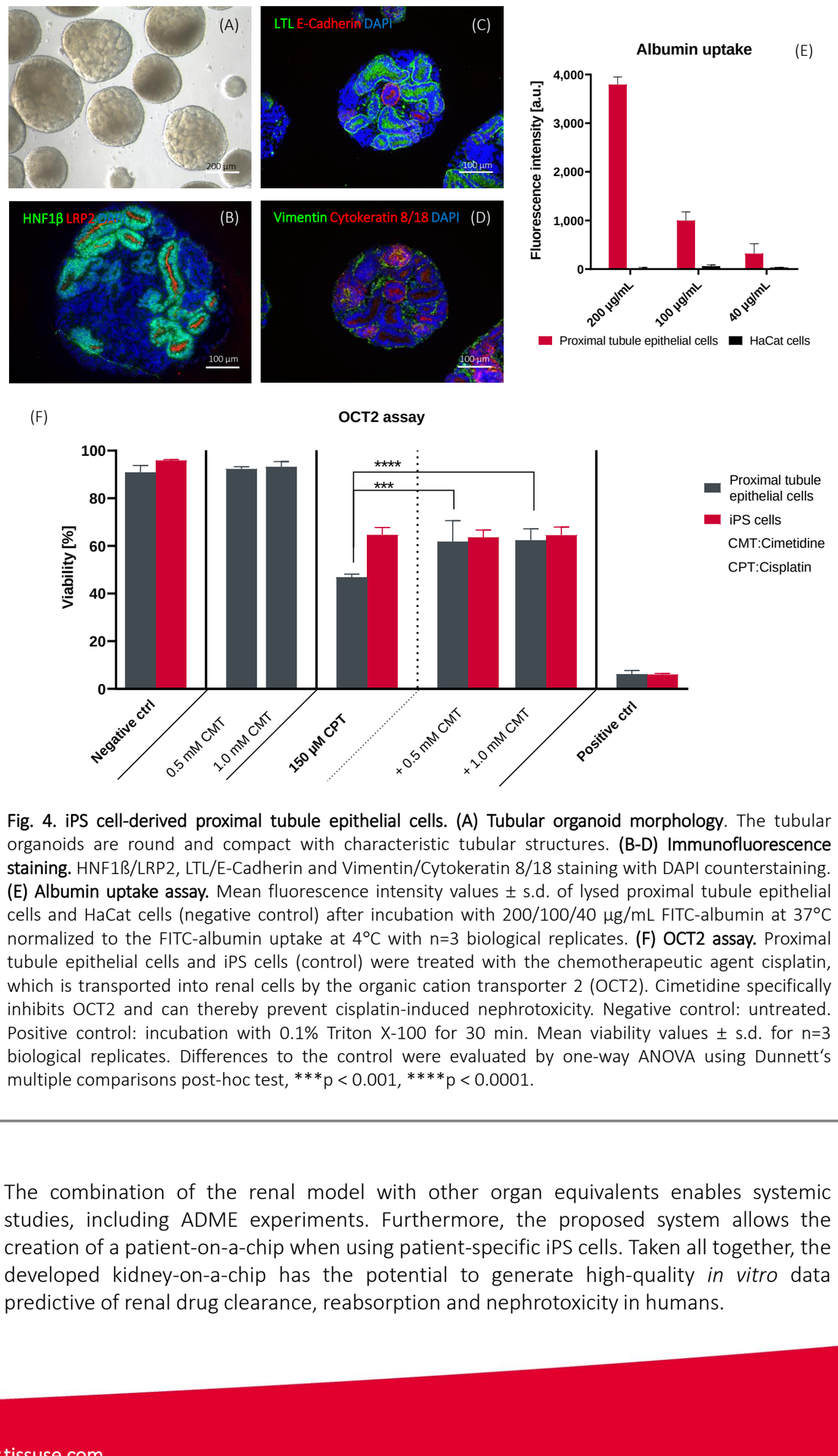

Fig. 4. iPS cell-derived proximal tubule epithelial cells. **(A) Tubular organoid morphology**. The tubular organoids are round and compact with characteristic tubular structures. **(B-D) Immunofluorescence staining.** HNF1ß/LRP2, LTL/E-Cadherin and Vimentin/Cytokeratin 8/18 staining with DAPI counterstaining. **(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. **(F) 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 elaborate safety, efficacy and nephrotoxicity studies, as wells as for mechanistic studies of renal development or disease.

The combination of the renal model with other organ equivalents enables systemic studies, including ADME experiments. Furthermore, the proposed system allows the creation of a patient-on-a-chip when using patient-specific iPS cells. 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.