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Technical Note

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Human iPS-derived renal tubule MPS for predicting drug transport and renal toxicity via OAT1/3 and OCT2

Drug excretion is mediated by transporters expressed in renal tubules. However, current in vitro models often exhibit insufficient expression of key transporters, such as organic anion transporters (OAT1/3) and organic cation transporters (OCT2), making it difficult to accurately evaluate renal excretion, pharmacokinetics, and drug-drug interactions.
In this study, we developed a renal MPS using proximal tubule epithelial cells derived from human iPS cell–based kidney tissue (iPS-RPTECs). iPS-RPTECs exhibited higher expression levels of OAT1/3 and OCT2 compared to immortalized RPTECs, enabling the evaluation of drug excretion and nephrotoxicity mediated by these transporters.

Experimental Method

Kidney tissue was generated from human iPS cells using a protocol established by our advisor, Dr. Minoru Takasato (currently Team Leader at RIKEN). iPS-derived renal proximal tubule epithelial cells (iPS-RPTECs) were then isolated via magnetic-activated cell sorting (MACS) and seeded onto MPS chips. Western blotting confirmed that iPS-RPTECs exhibited higher protein expression of OAT1/3 and OCT2 compared to immortalized cells (Fig. 1).

The MPS chip was fabricated from PDMS and consisted of top and bottom microchannels, each measuring 1 mm in width and height. These channels were separated by a porous PET membrane with 3 μm pores. The top channel was designated as the urine side, and the bottom channel as the blood side. iPS-RPTECs were seeded on the upper surface of the porous membrane. After formation of a confluent epithelial layer, drug transport was assessed. Test compounds were introduced into the bottom (blood side) channel, and the compounds transported by iPS-RPTECs into the top (urine side) channel were collected over time. The concentrations of compounds in the collected samples were quantified using LC-MS.

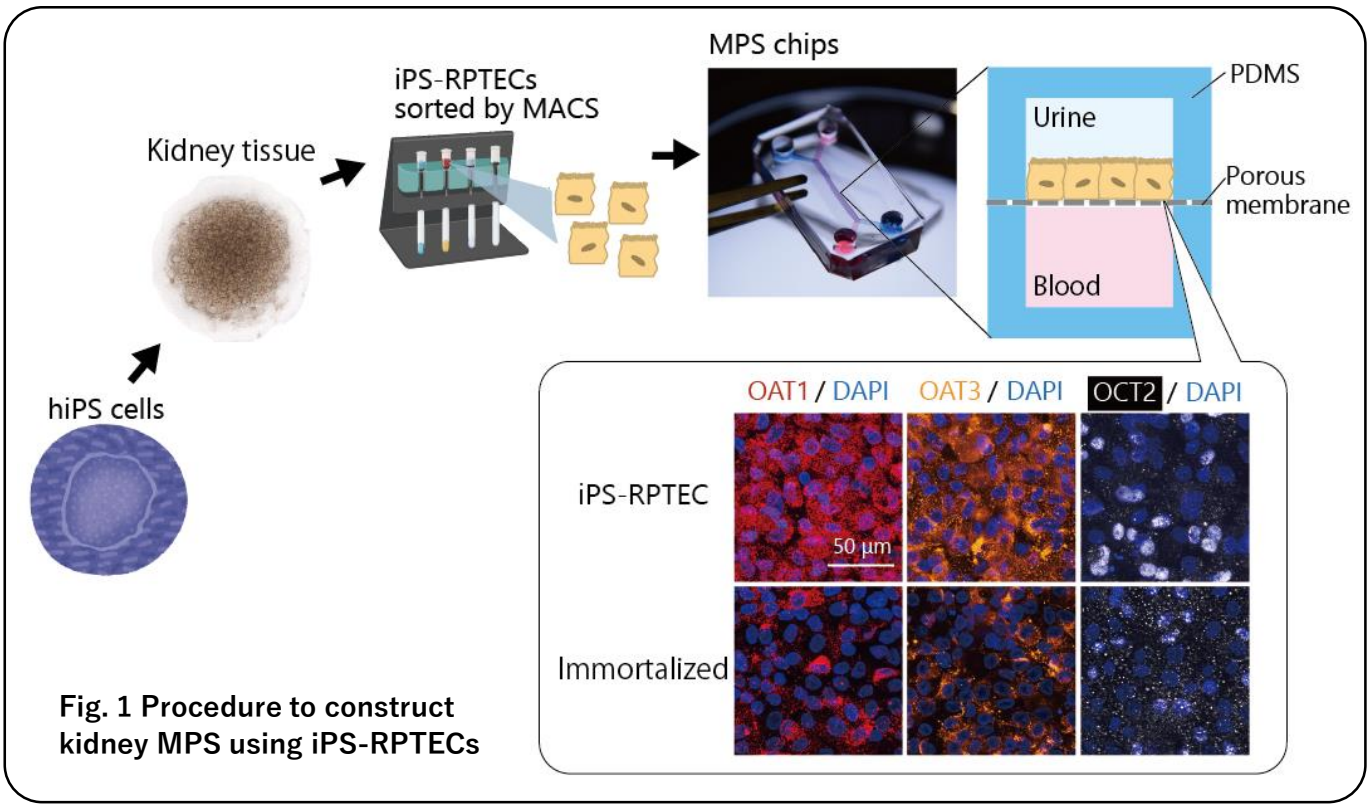

Fig. 1 Procedure to construct kidney MPS using iPS-RPTECs

Evaluation of drug transport via OAT1/3 and OCT2

Using MPS seeded with either immortalized RPTECs or iPS-RPTECs, we evaluated the transport of Adefovir (an OAT1 substrate), Rosuvastatin (an OAT3 substrate), and Metformin (an OCT2 substrate). These compounds are normally transported from the blood side (basal) to the urine side (apical) (Fig. 2a). To confirm the directionality of transport, we compared basal-to-apical (B-to-A) and apical-to-basal (A-to-B) transport.

In immortalized RPTECs, the transport rates of all three drugs were similar in both directions, indicating a lack of active transporter-mediated movement. In contrast, iPS-RPTECs showed significantly greater B-to-A transport than A-to-B transport for all three compounds, suggesting functional expression of relevant transporters (Fig. 2b).

To confirm whether Adefovir and Rosuvastatin transport was mediated by OAT1 and OAT3, respectively, we tested the effect of Probenecid, an OAT1/3 inhibitor. Treatment of iPS-RPTECs with 500 μM Probenecid markedly reduced the transport of both compounds, demonstrating effective inhibition of OAT1/3-mediated transport (Fig. 3).

In conclusion, our MPS platform using iPS-RPTECs enables the evaluation of drug transport via OAT1, OAT3, and OCT2.

Nephrotoxicity evaluation

Using iPS-RPTEC-based MPSs, we assessed the nephrotoxicity of cisplatin and aristolochic acid, which are taken up into renal cells via OCT2 and OAT1/3, respectively. Nephrotoxic effects were observed 24 hours after exposure to either compound. Notably, the introduction of cimetidine, an OCT2 inhibitor, effectively suppressed cisplatin-induced toxicity. These results demonstrate that iPS-RPTEC MPSs can be used to evaluate not only nephrotoxicity but also the protective effects of transporter inhibitors.

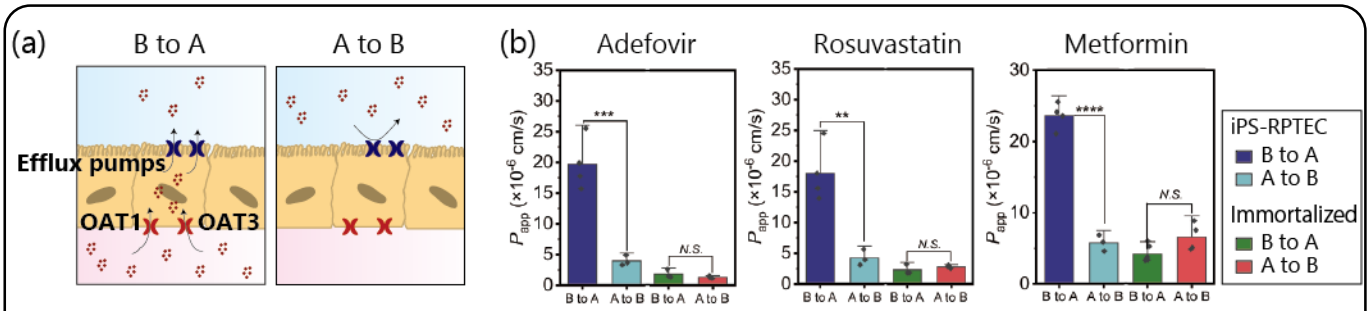

Fig. 2 Evaluation of drug transport using renal MPS

a. Illustration of the direction of drug transport. Drugs are uptaken into cells via OAT1/3 or OCT2 expressed on the basal side and expelled to the urinary side. When drugs are introduced on the urinary side, they are not actively transported.

b. Results of transport of Adefovir, Rosuvastatin, and Metformin. Vertical axis shows drug transport rate per unit area (P_{app})

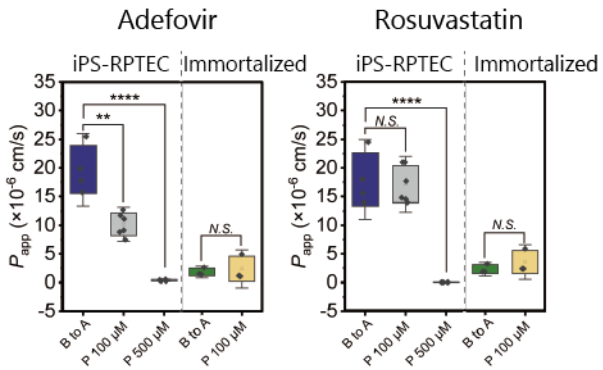

Fig. 3 Inhibition of drug transport by Probenecid

In iPS-RPTEC MPSs, the transport rate decreased as the concentration of Probenecid increases.

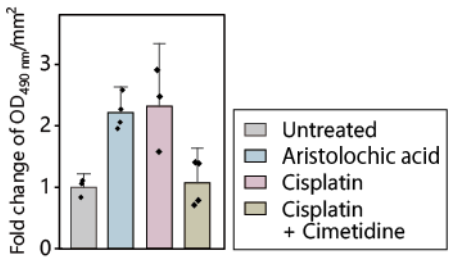

Fig. 4 Nephrotoxicity test using iPS-RPTEC-based MPS

After 24 hours of exposure, the amount of LDH released into the culture supernatant was measured using a fluorescence-based LDH assay kit. The vertical axis represents the fluorescence intensity.