Kidney

Development of highly-differentiated human primary proximal tubule MPS model (aProximate MPS™ Flow)

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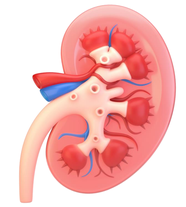

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Introduction

The proximal tubule (PT) is the key nephron segment mediating renal drug elimination and is the primary site of drug induced nephrotoxicity. However, current animal studies have proved poorly predictive of human outcome. To address this, there has been a recent upsurge in physiological relevant microphysiological systems (MPS) of PT to recapitulate differentiation and function in vitro. Here we present results from our recently developed aProximate MPS™ human PT platform (Patent No: G001336.GB), in which primary human PT cells are subject to fluidic media flow and a shear stress between 0.1-2 dynes/cm^2.

Methods

Computational Fluid Dynamics (CFD) was used to optimise uniformity of the shear stress within the flow chambers. Using CAD software and 3D printing, we were able to rapidly prototype different plates with different format to determine optimal performance for primary cells within the flowplate system. Primary PT cells were seeded on the underside of Corning ThinCert™ and incubated overnight. After flipping, the flowplate was placed on a rocking platform at angle θ allowing passive liquid levelling creating lateral flow without the use of a pumping system.

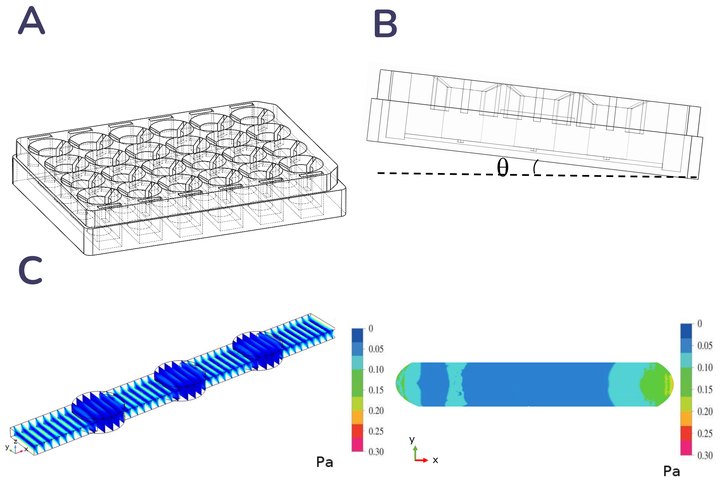
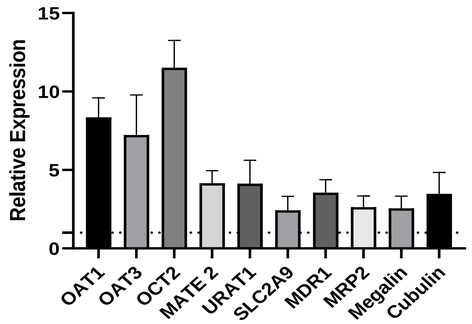


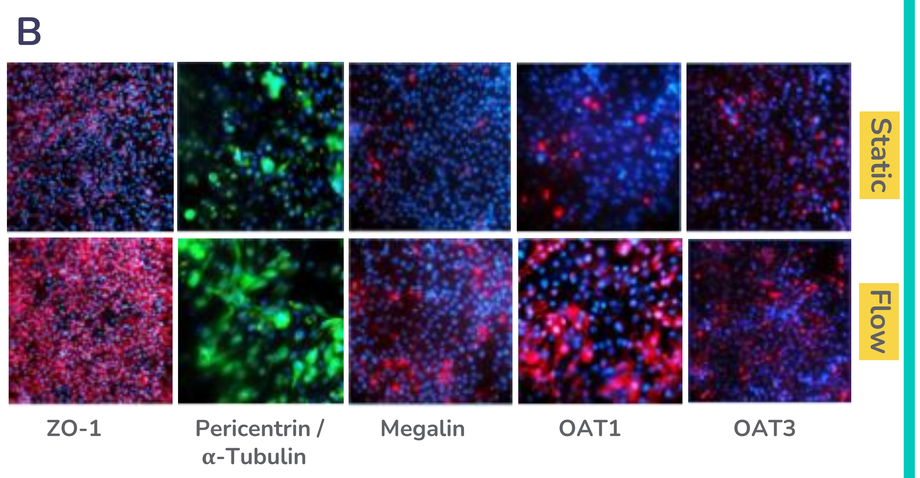
Figure 1. Design of aProximate MPS™ Flow system. A) Plate design in 24-well format, **B)** function of flowplate, **C)** CFD simulations of media flow within the channel showing uniform shear stress.

Results

qPCR was used to determined gene expression levels of key transporters involved in primary PT cells function.



IHC was used to confirm expression levels and to determine phenotypic differences between static and FSS-exposed cells.



Barrier function and integrity were assessed using TEER and Lucifer Yellow assays.

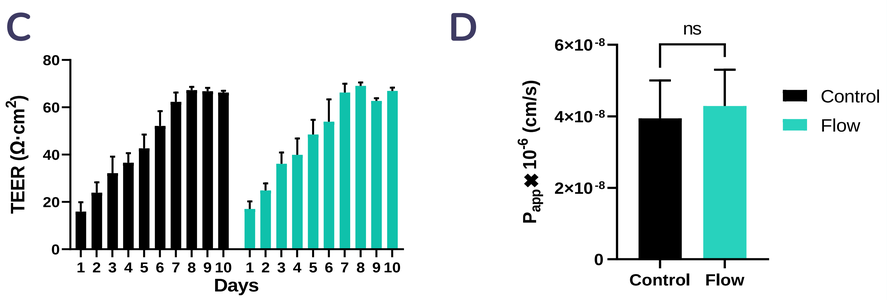
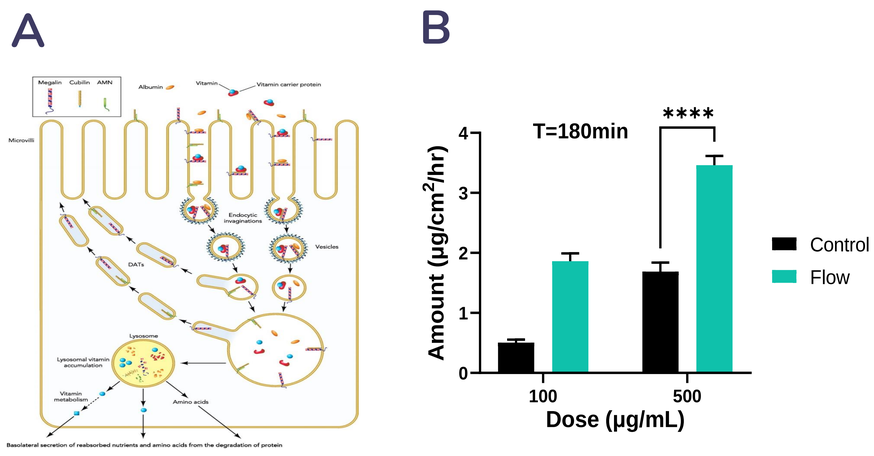
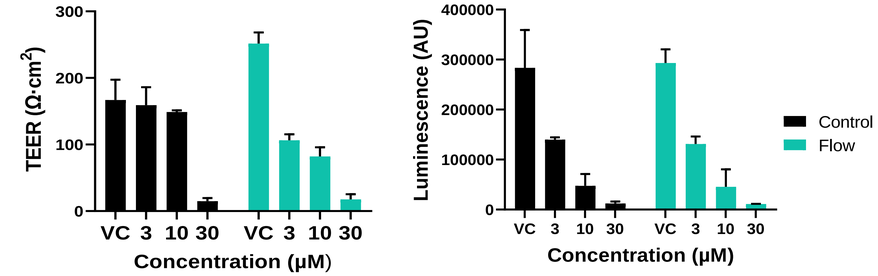


Figure 2. Characterisation and barrier function assessment. A) qPCR analyses of key transporters at D7 (n=5); **B)** IHC images of key proteins in human PT cells at D7; **C)** TEER measured from D1-D10; **D)** P_{app} measured using Lucifer Yellow up to 180 minutes (n=6).





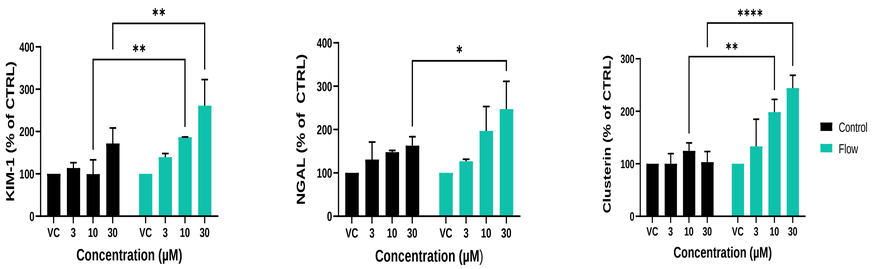


Figure 3. Functional Assays on aProximate MPS™ Flow. A) Illustration of Megalin and Cubulin cellular uptake. **B)** FITC-Albumin Uptake at 180 minutes; **C)** TEER measured at 72hrs following treatment; **D**) ATP release measured at 72hrs following cisplatin treatment; **E)** Renal injury biomarker release measured at 72hrs following treatment using MSD (n=3).

Conclusions:

This dataset, suggests that growing human PT cells in the MPS flow model, significantly improves phenotype and function and has significant benefit to the utility and near-physiology of the model.