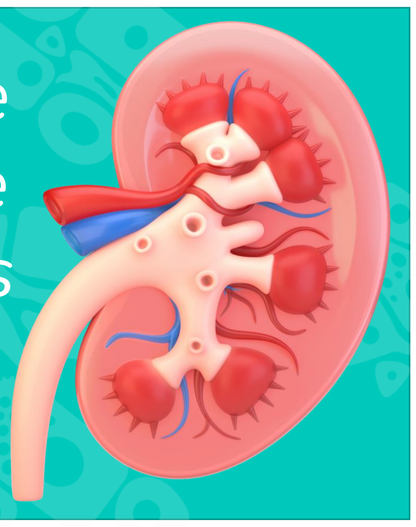
Developing predictive in vitro Glomerulus , Proximal Tubule and Loop of Henle renal cell model platforms to investigate the renal uptake and nephrotoxic liability of large molecules



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Introduction

Biologics represent a new modality of drug molecules which is rapidly expanding in market share. Several studies have demonstrated the biodistribution of siRNA and antisense oligonucleotide (ASO) is highly weighted to the kidney. Targeted uptake of siRNA and ASO by the renal tubule has been exploited as a mechanism to deliver specific siRNA and ASO to silence genes in the kidney. However, the non-specific nature of the interaction between large molecules and specific segments of the renal tubule has also generated off target nephrotoxicity. To better understand the interaction of large molecules (ASOs, siRNA, ADCs large peptides)with different segments of the tubule, we have developed novel primary cell culture models of the glomerulus /podocytes (GPCs) proximal tubule (PTC) and Loop of Henle (LOH) platforms to investigate both the mechanisms of large molecule interactions along the nephron.

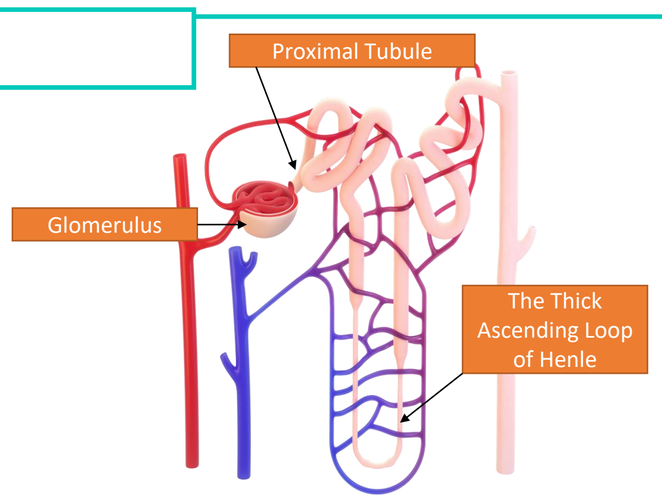


Figure 1: Generation of Human primary cell monolayers from Glomerulus, Proximal Tubule and Thick Ascending Loop of Henle(TALH). Cells from each segment were isolated by a collagenase digest –Percoll gradient and MACS sorting where appropriate. Cells were grown as monolayers on Transwell filter supports.

aProximate™ Glomerulus – Podocyte Effacement Assay

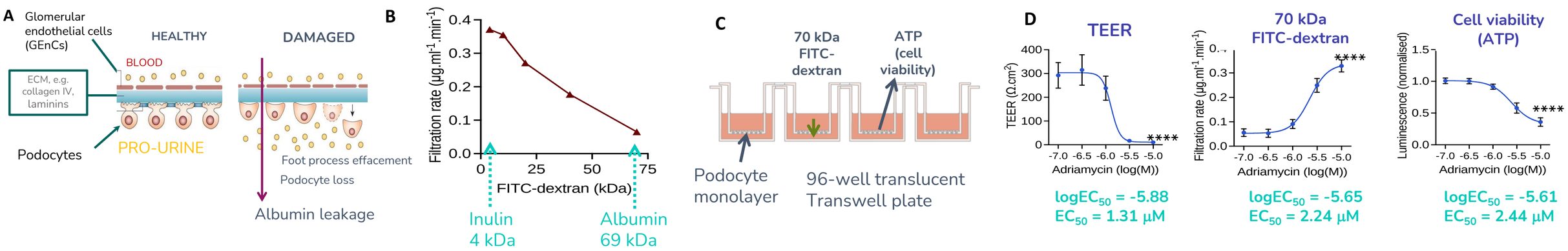
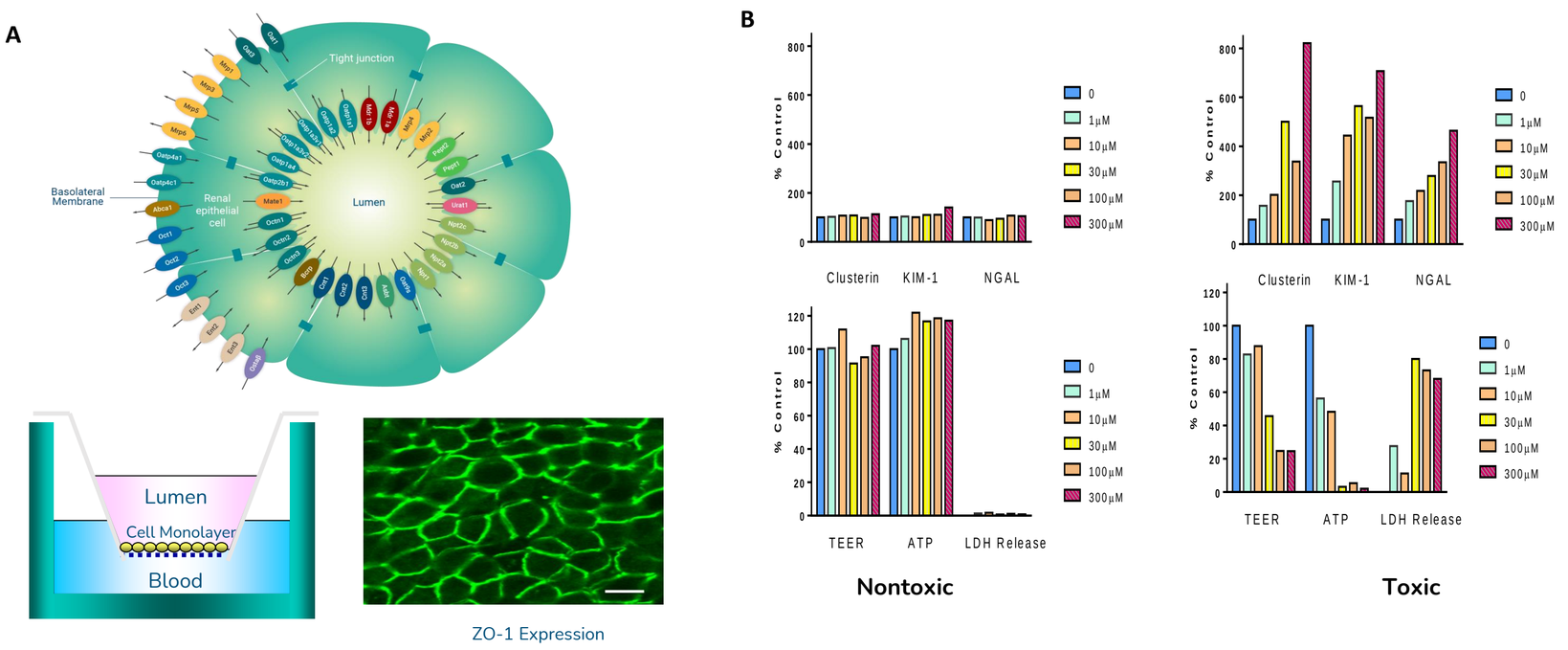


Figure 2 Glomerulus effacement assay A)Glomerulus consists of 3 layers; a glomerular endothelial layer, an ECM and specialised podocytes. The slit junctions formed between the podocytes pedicels wrapping around the capillaries provide the size and charge selective filter which prevents the leakage of large molecules into the filtrate, the barrier can be damaged by drug molecules resulting in an increased permeability to large molecules, B) Our *in vitro* podocyte model exhibits the same properties. C) Using a 70kDa FITC-Dextran we can quantify changes in permeability, D) The Adriamycin results in decreased TEER, increased 70KDa permeability and a decrease in viability of treated cells. Data is the mean and SEM of n=3 independent kidneys glomerular toxic agent

aProximate™ Proximal Tubule Nephrotoxicity model



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	Sens./Spec. (%)	PPV/NPV (%)
With all endpoints	70.0/62.5	70.0/62.5
Only biomarkers	70.0/87.5	87.5/70
> 2 biomarkers	60.0/93.8	92.3/65.2

Figure 3 aProximate™ Proximal Tubule cells are isolated from human kidney cortex and grown as monolayers on Transwell filter supports. A) The cells differentiate to recapitulate a monolayer expressing tight junctions and the polar distribution of transport proteins to the apical and basolateral membrane, reports have implicated that some large molecules damage the Thick Ascending Loop of Henle cells. B) To validate the cells as a predictive model of proximal tubule toxicity, cell monolayers were exposed to a range of 36 compounds with known toxicity profiles (19 nephrotoxic, 17 non-nephrotoxic). Experimental setup was a 72 hour challenge to 6 separate concentrations of each test compound. The end points of toxicity measured were release of the biomarkers; clusterin, KIM1 or NGAL together with changes in TEER, ATP content and LDH release. Data was analysed using a ROC analysis. The model had a sensitivity of 60% and a specificity of 94% with a positive predictive value of 93%. Error bars represent SEM (n=, 3 kidneys).

aProximate™ Thick Ascending Loop of Henle model

Figure 4: Large molecules disrupt monolayer integrity and increase paracellular permeability of TALH cells.
A) Recent reports have implicated that some large molecules damage TALH cells. To investigate this, we generated a human TALH model. The model was enriched in TALH markers Uromodulin; NKCC2 and ROMK and de-enriched in Aquaporin-1. Impact of TEER of treated cells normalized to control wells; control wells denoted by dashed line (100%). C) On challenge with a number of large molecules (ASOs or Peptides) reported to cause changes in TALH function, we could measure significant increase in Lucifer yellow permeability consistent with the concept of drug induced damage to TALH cells. P *,** and **** indicate p <0.05 and p <0.0001. Error bars represent SEM (n=3 kidneys).