KIDNEY

An MPS model of proteinuria for high throughput drug discovery

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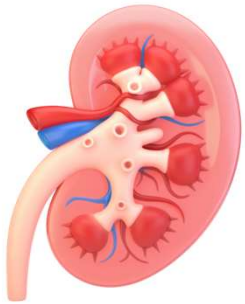

Abstract number

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Introduction

An early indication of kidney damage is the appearance of protein in the urine (proteinuria). In some cases the damage can resolve by itself, but in others a progressive loss of kidney function leads to Chronic Kidney Disease (CKD), for which there is no cure.

The glomerulus is the structure in the kidney that filters waste products and small molecules from the blood into the urine. Podocytes have long finger-like processes that wrap around the blood vessels of the glomerulus to form a size-selective sieve. Damage to these cells enlarges the size of the holes in the filter, leading to proteinuria.

Experiments to examine the causes of proteinuria use live rodents, and currently available *in vitro* models of glomerular cells are poor. In response to this, we have developed an MPS model of the glomerular filtration barrier (GFB) with proteinuria as a read-out (Fig. 1).

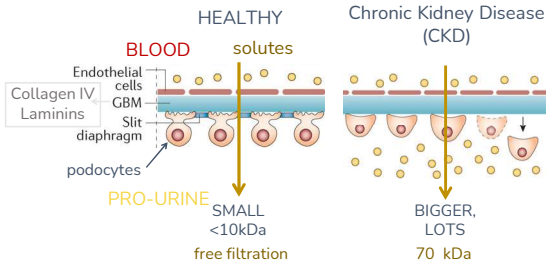


Figure 1: GFB structure and proteinuria assay principle. The assay monitors the rate of flux of FITC-dextran molecules of different molecular weights across the barrier *in vitro*.

Methods

Primary podocytes isolated from human kidneys (as ref. 1) were assessed for purity using flow cytometry (Fig. 2), and for key podocyte markers and central cilia (Fig. 3) by immunofluorescence microscopy. Cells seeded into 96-well Corning Transwell inserts were monitored for the formation of Transepithelial Electrical Resistance (TEER) using an EVOM2 Voltohmmeter. Duplicate or triplicate wells were administered with FITC-labelled dextrans (4, 10, 20, 40, 70 kDa), positively-charged (DEAE, 4, 40, 70 kDa) or negatively-charged (CM, 4, 40, 70 kDa), on the apical side of the membrane and the rate of flux determined following sampling of the basolateral compartment at 30, 60, 90 mins and performing a linear regression analysis (method adapted from ref. 2).

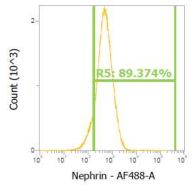


Figure 2: Podocyte population is ~90% pure. A fluorescent Nephrin antibody was used to determine population purity by flow cytometry.

Results

Monolayers of primary podocytes formed a TEER (Fig. 4) and displayed size (Fig. 5) and charge (Fig. 6) selectivity, comparable to the GFB *in vivo*. Administration of podocyte toxin Adriamycin disrupted the integrity of the monolayer (Fig. 7).

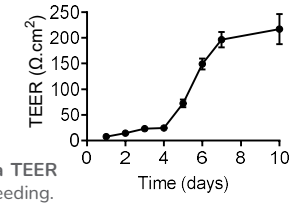


Figure 4: Confluent podocytes formed a TEER (right). TEER was monitored daily after seeding.

Size selectivity

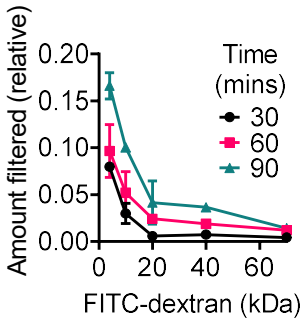


Figure 5: Our model exhibits size selectivity comparable to *in vivo*. Small FITC-dextran molecules moved through the barrier more easily than larger molecules, with a size cut-off of ~70 kDa.

Charge selectivity

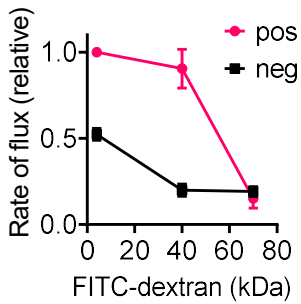


Figure 6: Our model demonstrates charge selectivity. Positively charged (DEAE) FITC-dextrans moved through the barrier more easily than negatively charged (CM) FITC-dextrans (*n*=3).

Barrier disruption by podocyte toxin

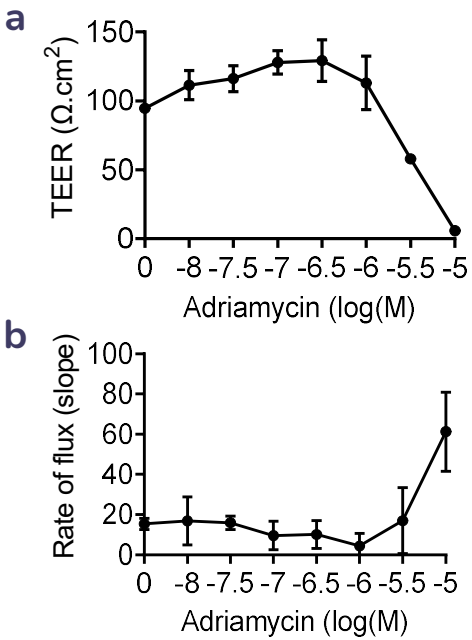
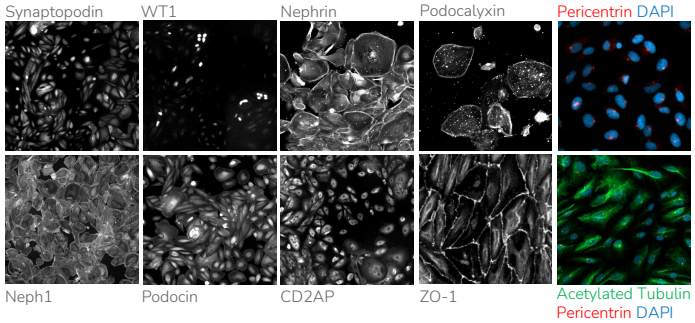


Figure 7: Podocyte toxin Adriamycin increases monolayer permeability to 70 kDa FITC-dextran. Podocytes were treated with various concentrations of Adriamycin for 72 hrs. **(a)** TEER values. **(b)** Barrier permeability to neutral 70 kDa FITC-dextran determined.

Figure 3: The podocytes express all of the key podocyte markers, including pericentrin and acetylated tubulin, markers of the central cilia.



Summary

- We have developed an MPS model of the Glomerular Filtration Barrier (GFB) using primary podocytes isolated from human kidneys.
- Podocytes grown on Corning Transwell inserts exhibit size and charge selectivity comparable to *in vivo*.
- The 96-well format makes our model amenable to high throughput drug screening and discovery applications.

References

1. Ni L *et al.* 2012 Nephrology 17: 525.
2. Hunt J L *et al.* 2005 J A S N 16: 1593.

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