ABSTRACT
We introduce a simple microfluidic device that mimics function of the kidney proximal tubule by integrating a polydimethylsiloxane (PDMS) microfluidic channel and human proximal tubule cells on a porous extracellular matrix (ECM)-coated porous membrane. This allows us to culture the kidney epithelial cells under a low level (0.2 dyne/cm²) of fluid shear stress that mimics that of the normal kidney tubule. By providing in this system, a more physiological microenvironment, we are able to enhance epithelial cell polarization, cytoskeletal reorganization, primary cilia formation and response to renal toxins. These results suggest that our system is useful for studying human-relevant renal toxicity, and has the potential for drug development and advanced tissue engineering applications.

KEYWORDS: human kidney proximal tubule cell, kidney-on-a-chip, nephrotoxicity, microfluidic device

INTRODUCTION
There is a great need for more predictive in vitro human kidney models for investigating absorption, distribution, metabolism, excretion, and toxicological properties of new chemical entities during the drug development process. Here we describe a biomimetic microsystem that reconstitutes critical functional aspects of the renal proximal tubule of the human kidney. To date, most studies on kidney function and nephrotoxicity have utilized conventional static two-dimensional culture systems [1]. However, both chemical and mechanical cues are critical for kidney function in vivo. The kidney filters about 180 liters of fluid per day, it is highly vascularised, and it regulates electrolytes, acid-base balance, and blood pressure to maintain our body’s homeostasis [2]. Microfluidic-based techniques provide powerful tools to recapitulate the complexity of the renal microenvironment in vitro, including fluid shear stress and transepithelial chemical gradients [3-5].

EXPERIMENTAL
To generate a microfluidic device, we microfabricated a sandwiched assembly containing a PDMS microfluidic channel (1 mm wide x 1 cm long x 100 μm high) apposed to a porous polyester membrane, and a PDMS reservoir for medium below; all layers were bonded after oxygen plasma treatment. Human kidney proximal tubule epithelial cells (Biopredic International) were cultured on the porous ECM-coated membrane within the microfluidic system and exposed to fluid dynamic forces that mimic the in vivo microenvironment (Fig 1).

Figure 1. Schematic of human kidney proximal tubule-on-a-chip in the form of multi-layer microfluidic device by integrating a PDMS microfluidic channel, a porous membrane, and a PDMS reservoir. Human primary proximal tubular cells were cultured on the device.
RESULTS AND DISCUSSION
Fluid shear stress was applied by controlling flow rate of culture medium through the PDMS channel and above the cells that were cultured on the ECM-coated membrane, with the PDMS reservoir below permitting analysis of transport between the lumen of the channel and the medium below. Culture of primary human proximal tubule cells under 0.2 dyn/cm² of fluid shear stress for 18 hours resulted in enhanced epithelial cell polarization, differentiated cytoskeletal morphology (corticle actin rings rather than stress fibers), and increased primary cilia formation (Fig 2). Renal drug transporter expression, specific markers of proximal tubule function, and effects of known nephrotoxic compounds were also analyzed using this microdevice (Fig 2 and 3).

Figure 2. Fluid Shear Stress Stimulates Kidney Cell Differentiation. In fluidic condition, cells are subjected to 0.2 dyn cm⁻² of shear stress for 18 hours. We investigated the effects of fluidic shear stress on cytoskeletal proteins (actin), tight junction-associated protein (ZO-1), marker of primary cilia (acetylated tubulin), and ion transporters (Na/K-ATPase and Na/HCO₃ cotransporter). Cell height was measured by confocal microscope images of MRP4 (apical marker protein) and Na-K-ATPase (basolateral marker protein) with x-z optical sectioned slices.

Figure 3. Cisplatin toxicity (a)TUNEL assay (b) KIM-1 assay (c) LDH assay
To evaluate the potential of this model to assess nephrotoxicity, we treated the cells with cisplatin (known human nephrotoxin) [6]. Since cisplatin toxicity is thought to be mediated via the human organic cation transporter 2 (hOCT-2), we also tested the ability to inhibit toxicity by treatment with the hOCT-2 inhibitor, cimetidine [7]. Multiple endpoints were used, including lactate dehydrogenase (LDH) assay for cell injury, TUNEL assay for toxicity due to apoptosis (TUNEL assay), and measurement of kidney injury molecule-1 (KIM-1) for kidney tubular damage (Fig 3). This biomimetic microsystem was able to recapitulate the \textit{in vivo} toxicity seen with cisplatin and its inhibition by cimetidine.

**CONCLUSIONS**

The data gathered thus far suggest that this “Human Proximal Tubule-on-a-chip” can provide a useful \textit{in vitro} model for studying renal physiology and pathophysiology. This novel system may also provide a useful and cost-effective tool for studying biotransformation profiles, renal pharmacology, renal drug transport and toxicity relevant to the human kidney, and hence help to facilitate the drug development process with a more human-relevant model.

**REFERENCES**


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