Investigating the potential of a kidney microphysiological system (MPS) to assess toxicity associated with exposure to chemicals

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Abstract

The need to reduce, refine and eventually replace animal use in toxicological studies has created an urgent need to improve current *in vitro* kidney models with systems that can be better predictors of human nephrotoxicity induced by hazardous chemicals. Microphysiological systems using human cells in a three-dimensional (3D) microenvironment are promising *in vitro* models with superior physiological relevance than the conventional 2D models to bridge translational gaps. The proximal tubule (PT) is the nephron segment that mediates secretion and reabsorption of xenobiotics and hence, is the primary site for renal injury. This study was designed to evaluate the potential of a proximal tubule MPS (Nortis PT-MPS) for risk assessment of renal exposure to chemicals. The Nortis MPS comprises chip designs that allows us to assess PT exposure to chemicals through luminal or basolateral sides, which are more consistent with human exposure. The tubules are fully formed and ready for testing in 10 days and samples can be collected as early as 1h after exposure to chemicals. In this initial phase we characterize the human immortalized proximal tubule cells used in the system. Cells are cultured in a 2-dimensional (2D) milieu and in the 3-dimensional PT-MPS and the expression of crucial receptors and drug transporters is assessed by immunofluorescence (IF) staining. Tubular formation and cell viability in the PT-MPS is appraised by IF and light microscopy. Initial experiments to assess Polymyxin B toxicity in the PT-MPS after 6h, 24h and 48h exposure showed increased cell death and release of biomarkers of injury. This new approach may allow us to obtain more accurate data on short- and long-term renal exposure to toxicants and detect biomarkers that provide rapid information on a specific context of use for risk assessment of kidney toxicity associated with food contaminants. This study is in accordance with the Division of Toxicology (DT) strategic plan to develop and expand *in vitro* models to provide more relevant and predictive data for human safety assessment of chemical hazards in foods and dietary supplements.

Introduction

Although animal toxicity testing has been the hallmark of drug safety studies, there is a growing consensus that animal models are poor predictors of human toxicity. Due to the lack of consistency of data obtained with current models, there is a critical need for development of robust, predictable *in vitro* models that replicate human physiology and provide relevant data to reduce, refine and eventually replace animal studies.

One of the main functions of the kidney is to eliminate impurities and chemicals from the body. The proximal tubule (PT) is the nephron segment that mediates secretion and reabsorption of xenobiotics and hence, is the primary site for injury. Emerging technologies, including microphysiological systems (MPSs), employ human renal epithelial cells in a 3-dimesional (3D) environment, to form a proximal tubule that recapitulates *in vitro,* the architecture and function of a human proximal tubule. The controlled, unidirectional flow of media allows to assess exposure to chemicals through luminal or basolateral sides of the proximal tubule, which is more consistent with human exposure. The cells in the MPS devices (chips) are viable for longer periods of time compared to traditional cell culture, which is suitable for short-term and long-term exposure assessment of chemicals.

This research will evaluate the potential of a kidney proximal tubule (PT) microphysiological system (MPS) using human cells as a new alternative model to assess toxicity of food chemicals of concern.

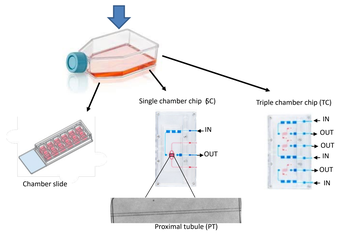
The first steps to acquire this information are: characterize the human PT cells used in the Nortis MPS system and assess viability, integrity and responses of the PT-MPS when exposed to a known nephrotoxic agent, Polymyxin B.

Mission Relevance

- ❑ Fulfill the Division of Toxicology's Strategic Plan to apply new approach methods (NAMs) to generate data with significant human concordance for predictive regulatory toxicology.
- ❑ Support FDA's mission to protect the public health by ensuring the safety of our nation's food supply and dietary supplements.

Materials and Methods

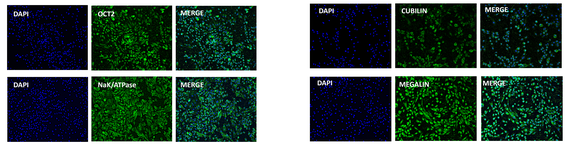
- Human proximal tubule cells (RPETC/TERT1, ATCC CRL-4031)



- Immunofluorescence staining (IF) of PT drug transporters and membrane proteins, in 2D cell culture (chamber slide) and in 3D PT-MPS (SC chips)
- Assessment of toxicity induced by Polymyxin B (50µM and 100 µM) after perfusion of PT-MPS for 6h, 24h, 48h (TC chips) at 1µL/min. Creatinine (50µM and 100 µM) used as a negative control for toxicity. Parameters analyzed:
  - ✓Live/dead cell viability assay, at 48h (IF)
  - ✓Lactate dehydrogenase (LDH) release in chip effluents at 6h, 24h, 48h
  - ✓Biomarkers of kidney injury in chip effluents at 6h, 24h,48h

Results

A. RPTEC/TERT1 cells in 2D cell culture



B. Proximal tubules in PT-MPS chips

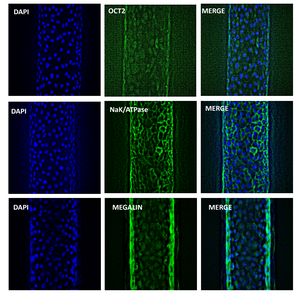
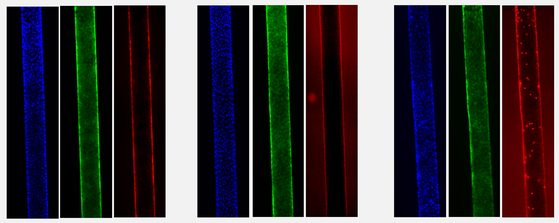


Figure 1. Immunofluorescence (IF) staining of the organic cation transporter OCT-2, the receptors megalin/cubilin and the membrane protein Na-K-ATPase pump in RPTEC/TERT1 cells. **A.** RPTEC/TERT1 cells grown in a traditional 2D culture; **B.** PT formed inside single chamber (SC) PT-MPS chips. Magnification 200x.

Results

A. Live (green) and dead (red) cell staining in PT-MPS 48h after exposure



B. Semi-quantitative dead cell count in PT-MPS 48h after exposure

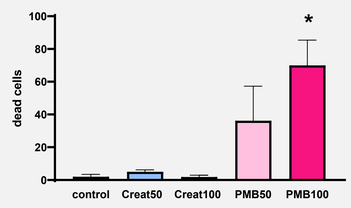


Figure 2. A. Representative IF pictures of live (green) and dead (red) cell staining in PT-MPS 48h after perfusion with medium only (control), Creatinine (50, 100 µM-negative control) or PMB (50, 100 µM). Magnification 40x. **B.** Semi-quantitative dead cell count performed with live/dead IF staining assay 48h after exposure. *P<0.01 vs all groups.

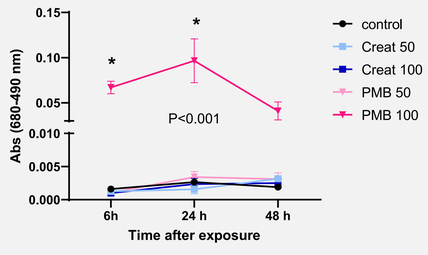
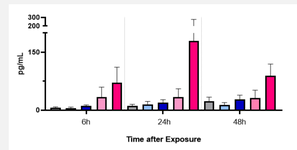
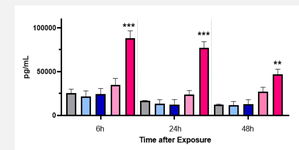


Figure 3. LDH release measured in chip effluents from PT-MPS after perfusion with medium only (control) , Creatinine (50, 100 µM-negative control) and PMB (50, 100 µM) for 6h, 24h and 48h. * p<0.05 vs PMB100 at 48h. P<0.001 PMB100 at all time-points vs other groups.

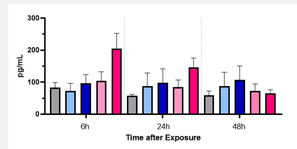
A. KIM-1



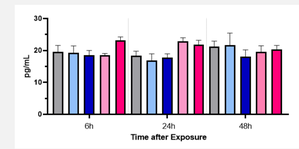
B. Osteoactivin



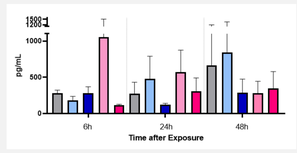
C. VEGF



D. Calbindin



E. Clusterin



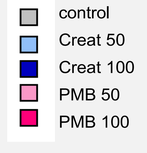


Figure 4. Biomarkers of kidney injury measured in PT-MPS effluents after perfusion with medium only (control), Creatinine (50, 100 µM-negative control) or PMB (50, 100 µM). ***P<0.001 compared to other groups at the same time exposure.

Summary and Conclusion

- ✓ Initial IF staining assay showed that RPTEC/TERT1 cells expressed OCT-2, megalin/cubilin and Na-K-ATPase, either in 2D traditional cell culture or in the 3D PT-MPS (**Figure 1**). These proteins are typically expressed in PT of human kidney.
- ✓ Perfusion of PT-MPS with PMB resulted in relatively higher number of dead cells compared to control and creatinine-perfused PT-MPS (**Figure 2**). PMB is taken by PT cells through the megalin/cubilin complex.
- ✓ LDH release in effluents of PT-MPS was significantly higher after perfusion with PMB100 µM, suggesting cellular damage.
- ✓ There was an increase trend in KIM-1 and VEGF **(Figure 4, A and C),** while osteoactivin was significantly higher as early as 6h in effluents of PT-MPS perfused with PMB 100 µM (**Figure 4,B**). Calbindin, a distal tubule marker of injury, was not altered in the PT-MPS by the tested compounds (**Figure 4,D**).

While additional testing is needed, these preliminary results demonstrate the potential of a PT-MPS system as a new *in vitro* approach method to assess nephrotoxicity of different chemicals.