Assessing Cisplatin-induced nephrotoxicity using both conventional cell cultures and a novel microphysiological system

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Abstract
Cisplatin is used as a chemotherapy agent against aggressive cancers. While Cisplatin remains as an effective therapeutic for treating cancer, the kidneys remain a primary target for damage. The nephrotoxicity of Cisplatin was evaluated in this study using LLCPK-1 cells, fetal proximal tubule epithelial cells (PTEC), and adult PTECs. Initial experiments were performed in 2D cell culture and further evaluated in the 3D microphysiological system (MPS). The MPS are being used as a model of cell culture ex vivo and previous developments have shown their utility to mirror the human body in regards to the proximal tubule of the kidney. We compared the cellular responses to different concentrations of Cisplatin, using both traditional 2D cell culture techniques as well as the 3D MPS. In 2D cell culture LLCPK-1 cells were the most sensitive cells to Cisplatin as well were more sensitive to Cisplatin in the 3D MPS. The Kelly lab believes successfully modeling nephrotoxicity using the MPS will translate towards a fully-functioning human model on a chip. The human model on a chip will not only limit the use of animal testing, but serve as a preclinical system to assess the human cellular response to new chemical entities.

Introduction
• Cisplatin is a cytotoxic drug used to treat aggressive types of cancers.
• Cisplatin is cleared by the kidney by both glomerular filtration and tubular secretion.
• Nortis has developed a novel technology for generating small segments of human tissues and organs via microfluidic chips.
• Three-dimensional culture is achieved by injecting proximal tubule epithelial cells into the tubular voids of the chip.
• PTECs were cultured in the 3D MPS and exposed to physiological relevant serum concentrations of Cisplatin and toxicity was assessed.

Methods
Cell isolation
Cortical tissue was finely minced, added into collagenase, resuspended, and transferred to sterile conical tubes. The tissue was incubated in a shake incubator for 30 min. The tissue was transferred to conical tubes containing horse serum, and vortexed. Cells were centrifuged, and the pellet was resuspended in 10 ml of DMEM and plated into a 2D culture flask.

Cell plating
Upon reaching confluency, cells were trypsins digested, collected, and pipette mixed in media. Cells were counted and resuspended in medium to the desired cellular concentration and plated into a 96-well plate.

Dosing with Cisplatin (2D)
Serial dilutions were performed across a concentration range spanning 30 wells in the 96-well plate. Cells were dosed with varying concentrations of Cisplatin (500µM to 0µM) for 24 hours.

Viability assessment (2D)
Absorbance of each well in the 96-well plate was analyzed using a plate reader (abs 570nm). An MTT cell proliferation assay was used on the Cisplatin-treated LLCPK-1 cells. 10µl of the MTT solution were added to each well and incubated for 4 hours. After incubation, 100µl of SDS-HCl solution were added to each well and incubated. Absorption was read at 570nm and viability was calculated relative to the control (0µM) cells.

Device preparation and PTEC culturing
Device matrices were sterilely injected with Collagen type I and incubated overnight. The devices were coated with a Collagen IV/fibronectin solution for cellular attachment. Isolated PTECs from 2D culture were injected into the MPS using a syringe, incubated overnight, and fluidic culture was achieved using media-filled syringe pumps.

Viability assessment
Viability of the PTECs in the MPS was assessed using cellular stains from a live/dead kit. Viability was assessed by comparing the degree of cellular stain in the presence and absence of Cisplatin. Calcein AM fluorescently demonstrates a green signal in contrast to dead cells that will quench the “dead” dye, ethidium bromide, which demonstrates a red signal.

Results

Figure 1. Flowchart of cellular isolation, culturing, and the MPS.

Figure 2. Assessment of Cisplatin-induced toxicity in traditional cell culture. LLCPK-1 cells, fetal PTEC, and adult PTECs were plated in a 96-well plate and dosed at concentrations of Cisplatin ranging from 500µM to 0µM. Photos were taken after 24 hours of exposure and cellular morphology was compared.

Figure 3. Establishment of Cisplatin EC50. Using LLCPK-1 cells plated in 96-well plate, cells were dosed at concentrations of Cisplatin ranging from 500µM to 0µM and viability was assessed using MTT assay. EC50 value was estimated using GraphPad software.

Conclusion
• Fetal PTECs appeared to be more sensitive to Cisplatin-induced injury based off their altered morphology, but assessment of viability revealed LLCPK-1 cells to be more sensitive to fetal/adult PTEC (data not shown).
• No significant signs of toxicity were observed when PTECs cultured in the 3D MPS were exposed to physiologically relevant concentrations of Cisplatin (25µM) relative to control for 24 hours.
• PTECs that sporadically grew throughout the tubule appeared to be more sensitive to Cisplatin exposure.

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