A 3D Human Kidney Nephrotoxicity Model

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Abstract
The proximal tubule is a major target for drug toxicity that needs to be evaluated during the drug development process. Renal proximal tubule epithelial cells (RPTEC; Sigma-Aldrich SA7K clone MTOX1030) grown as perfused tubules against an ECM were cultured in the 3-lane system of the OrganoPlate®. They formed up to 40 leak-tight tubules with access to the basolateral as well as the apical side of the epithelial cells. Exposure to nephrotoxicants caused a dose-dependent disruption of the epithelial barrier, a decrease in viability, an increase in effluent LDH activity, and with cisplatin, increased expression of the DNA-damage marker H2AX, as detected by immunostaining. Our results demonstrate that we developed a functional 3D perfused proximal tubule model with advanced renal epithelial characteristics that can be used for drug screening studies for kidney toxicity.

Materials and Methods
Sigma-Aldrich SA7K cells
SA7K cells are a pseudo-immortalized human renal proximal tubule epithelial cell line that was developed from primary cells using CompoZr® Zinc Finger Nuclease technology. The cells were engineered to avoid cellular senescence and thus enable extended population doublings. SA7K cells exhibit normal epithelial morphology, express characteristic proximal tubule markers (e.g. CD-13 and α-GST), and carry out typical proximal tubule functions including albumin uptake, γ-glutamyl transpeptidase activity, increased secretion of cAMP in response to parathyroid hormone, and functional activity of both uptake and efflux transporters.

Highlights
• Functional human kidney-on-a-chip model
• Assess the effects of nephrotoxic compounds on 40 parallel cultured renal tubules formed using human RPTECs (SA7K)
• Multiplex non-invasive (viability) assays in a microfluidic format
• OrganoPlate® is compatible with most plate readers, microscopes, and robotic handling equipment.

Mimetas 3-lane OrganoPlate®

Figure 1. Mimetas 3-lane OrganoPlate®. a) Modified bottom of a standard 384-well microtiter plate with the microfluidic channels embedded between glass plates. b) Schematic of a chip with 3 lanes. c) 3D rendering of the tissue area of the 3-lane chip. The left lane comprises the RPTEC tubule with apical perfusion, the middle lane the ECM and the adjacent right lane functions as a perfusion lane (vascular channel).
Mimetas 3-lane OrganoPlate®
For all experiments a 3-lane OrganoPlate® (Mimetas BV, 4003 400B) with a channel width of 400 µm and a height of 220 µm was used. The top part of this plate is a standard 384-well microtiter plate with a modified glass bottom with 40 microfluidic chips embedded. Each chip consists of three channels separated by ridges, the phaseguides (fig. 1).

OrganoPlate® culture
An extracellular matrix (ECM) gel composed of 4 mg/mL collagen I, 100 mM HEPES and 3.7 mg/mL NaHCO3 was injected to the middle lane of the 3-lane chip. The OrganoPlate® was placed in 37°C incubator for 20 minutes to allow for polymerization of collagen. HBSS was added and the plate was incubated overnight at 37°C. SA7K cells were seeded to the adjacent lane and placed in 37°C incubator on an angle to allow for adherence of cells to the collagen I. After attachment, RPTEC Complete Medium (Sigma-Aldrich, RPTEC Complete Supplement MTOXRTSUP + DMEMα M4526) was added and the plate was placed on a rocker platform enabling bidirectional flow through the channels by passive leveling. Formation of tubules occurred over 6 days of growth.

Nephrotoxicity Assay
At day 6 in culture, cells were exposed to concentration ranges of the nephrotoxins cisplatin (Sigma-Aldrich, P4394), gentamycin (Sigma-Aldrich, G1397), diglycolic acid (Sigma-Aldrich, 143073), and potassium dichromate (Sigma-Aldrich, 1048640) prepared in RPTEC Tox Medium (MTOXRTSUP + DMEMα M4526). The following endpoints were measured after 48-hour exposure: Barrier integrity, cell viability (WST-8, Sigma-Aldrich, 96992), Lactate Dehydrogenase (LDH) activity in supernatant (Sigma-Aldrich, MAK066) and DNA damage for marker H2AX (Cell Signaling Technology, 9718S).

Figure 2: Proximal Tubule-on-a-chip model in the 3-lane OrganoPlate® platform. a) Seeding SA7K cells against collagen I: After loading collagen I into the middle channel cells were seeded in the adjacent channel. By gravity cells are triggered to attach to the gel. After inducing flow cells start growing a perfused tubular structure. Dimensions of the channels are in µm. b) Phase contrast images at day 0, day 4, and day 6 after seeding of SA7K cells. Images show that the cells form a tubular structure in the top channel in 6 days. Scale bar = 200 µm. c,d) 3D reconstruction images of SA7K tubules in the OrganoPlate® showing a view into the lumen of tubules (apical side). The magnification shows a single z-slice of the cells growing against the ECM. Nuclei in blue. Scale bars = 30 µm. Adapted from Vormann et al 2018.
Figure 4: Viability loss and increased LDH activity after 48-hour exposure to nephrotoxicants. **a-d)** To quantify viability of the cells a WST-8 assay was used. WST-8 is reduced by the cells to an orange formazan product which can be measured with an absorbance reader at 450 nm directly in the OrganoPlate®. Viability was significantly reduced (p<0.5) by all four nephrotoxicants in a concentration-dependent manner compared to vehicle control. **e-h)** LDH activity in the medium represents the amount of cell death. The LDH assay detected, similar to the WST-8 assay, had a significant effect for all four compounds. Adapted from Vormann et al 2018.
Results:

**SA7K cells form polarized tubules in the OrganoPlate®**

SA7K cells were seeded into the Mimetas OrganoPlate® as described above and tubule formation was followed over six days. Figure 2b shows optimal tubule formation of SA7K cells over this time period. Antibody staining against ZO-1 (tight junction protein located on cellular borders), acetylated tubulin (located on primary cilia pointing towards the lumen of the tubule), and ezrin (expressed on the apical surface) confirmed the correct polarization of the renal tubules (fig. 2c-d).

**SA7K cells form tight barriers in the OrganoPlate®**

The integrity of the epithelial barrier was investigated using fluorescently-labeled dextrans of 4.4 kDa and 150 kDa that were administered to the lumen of the tube. Leakage of the fluorescent dextran from the perfusion channel into the gel compartment was monitored and quantified in order to have a measure for the integrity of the epithelial cell monolayer (fig. 3). Subsequently, the apparent permeability ($P_{app}$) could be calculated.

**Nephrotoxicity Assessment in the Proximal Tubule-on-a-Chip**

Kidney tubules were assessed for toxicity response to four known nephrotoxicants - cisplatin, gentamycin, diglycolic acid and potassium dichromate. Six days after seeding, the tubules were exposed to test compounds for 48 hours. Each compound was tested over a range of at least six concentrations. The toxic effect on the proximal tubules was determined through multiplexing several assays: barrier integrity, cell viability and LDH release. In addition, immunohistochemical staining (IHC) was used to detect cisplatin-induced DNA damage.

All four compounds showed concentration-dependent disruption of the barrier integrity of the tubules as determined by leakage of fluorescent dextrans (4.4 kDa and 150 kDa) into the adjacent ECM (fig. 3 b-f). The disruption of barrier integrity was closely correlated with leakage of LDH into the luminal perfusate and with loss of cellular viability for each toxicant (fig. 4). The observed IC$_{50}$s for each toxicant in this 3D multiplexed assay were similar to values reported in the literature using primary human proximal tubule cells (Landry et al. 2011, Li et al. 2014).

Cisplatin is an anticancer drug that is known to cause DNA damage through alkylation. IHC staining was carried out to detect DNA damage using an H2AX antibody (fig. 5). A concentration-dependent increase in DNA damage was observed over a concentration range of 5 to 270 µM. Interestingly, an effect was detected at the lowest cisplatin concentration tested (5 µM), compared to the live cell assays where adverse effects were not seen until 30 µM.

![Image of immunofluorescent staining](image_url)
Conclusion

We describe the development of a functional proximal tubule-on-a-chip model which can be implemented for routine assessment of kidney toxicity. The OrganoPlate® platform allows parallel culture and assessment of 40 independent kidney tubules. This is important in toxicological studies or in compound testing as it allows for proper controls, replicates and serial dilutions. Induction of flow is realized by passive leveling on an interval rocker system, which is an easy-to-use alternative to complex pump solutions. Other than the rocking platform, no further specialized lab equipment is needed. The platform is pipet accessible and necessary media changes or reagent additions are non-invasive. This new model provides a powerful example of the ability to multiplex multiple non-invasive assays in a microfluidic format.

The barrier integrity can be monitored in real time by adding a reporter dye. In parallel, cellular viability (WST-8 assay) and enzyme leakage (LDH release) were also measured. All three assays yielded a similar result at a given exposure for the panel of nephrotoxicants, indicating the robustness of the model. Immunohistochemical staining for the assessment of DNA damage (H2AX) was used as an addition to the live assays.

In summary, we developed a user-friendly, functional proximal tubule-on-a-chip model that can be used to study the effect of compounds in 40 parallel cultured renal tubules. The functionality of the platform in combination with the ease of handling and moderate throughput makes this an excellent platform for studying nephrotoxicity.

References