3-D Human Renal Tubular Organoids Generated from Urine-Derived Stem Cells for Nephrotoxicity Screening

Haibin Guo∇, Nan Deng∇, Lei Dou, Huifen Ding, Tracy Criswell, Anthony Atala, Cristina M. Furdui and Yuanyuan Zhang

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## ASSOCIATED CONTENT

**Supporting Information**.

Figure Legends

**Figure S1**. Optimization of 3D organoid size and cell viability. a) The size of the organoids increased with increased seeding density (from 1,000 to 8,000 cells) after 7 days of culture as viewed using phase-contrast microscopy (scale bar 100 μm); b) Paraffin sections of 3D organoids were stained with H&E for internal morphologic characterization. Tubular-like structure formation in organoids with 4,000 cells (arrows); c) Organoids were stained using Life Technologies LIVE/DEAD Cell Imaging Kit (live cells = green, dead cells = red) and imaged using an Olympus FV10i confocal microscope (bottom panels, I–IV). Scale bars represent 100 μm; d) Cell Viability of human USC 3D organoids at different numbers of initial seeding cells one after week culture, assessed by Live/Dead cell imaging kit e) Quantification of organoid size over time (n = 6). Data presented as mean ± SD. Significance: *p < 0.05.

**Figure S2**. Tubular structure formation within organoids composed of USCs two weeks after exposure to k-ECM. **a**). Paraffin sections of USC-derived organoids (arrows) stained by H&E (low and high magnification); **b**). Quantification of tubular structure formation in 3D culture (n = 6). Data presented as mean ± SD. Significance: *p < 0.05.

**Figure S3**. USCs exposed to k-ECM were differentiated into renal cells after 14 days in 3D culture. **a**). Confocal images of human USC derived organoids (scale bar, 100 μm). Induced USC (4,000 cells at *p3*) expressed the renal tubular epithelial cell marker (AQP1) and podocyte markers (nephrin and synaptopodin, *i.e.* SYNPO); **b**). Quantification of cells that expressed renal cell markers in 3D culture (n = 6). Data presented as mean ± SD. Significance: *p < 0.05.

**Figure S4**. Cisplatin and acetone-induced cytotoxicity of induced-USC (*p3*) organoids 3 days after treatment. **a**). USCs in 3D culture displayed semi-transparency around the edges of organoids as seen by phase contrast microscopy and cell structure was well retained as seen by H&E staining (left column) (scale bar, 50 μm). Organoids showed signs of apoptosis or necrosis when exposed to 1% acetone. About 2/3 of the cells displayed some degree of nuclear dissolution (karyolysis) and/or nuclear fragmentation in H&E stained sections (middle column). Almost all cells in the 3D organoids appeared dark with loss of the semi-transparency seen in untreated organoids by phrase contrast microscopy. Nuclei condensation or pyknosis indicated cell death or necrosis when exposed to cisplatin (200 μm/ml) (right column); **b**). KIM-1 and **c).** CYP2E1 expression following drug exposure to cisplatin and acetone for 3 days (n = 6). Data in graphs presented as mean ± SD.

## AUTHOR INFORMATION


### Corresponding Author

*Phone: 1-336-713-1189 (office).

E-mail: yzhang@wakehealth.edu

ORCID

Yuanyuan Zhang: 0000-0002-0050-0051


### Author Contributions

∇Haibin Guo, and Nan Deng contributed equally to this work.

### Notes

The authors declare no competing financial interest.

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## ABBREVIATIONS

ATN, Acute tubular necrosis; RTEC, Renal tubular epithelial cells; USC, human urine-derived stem cell; k-ECM, kidney extracellular matrix; SYNPO, synaptopodin; KIM-1, kidney injury markers molecule-1; KSFM, keratinocyte serum free medium; DPBS, Dulbecco phosphate-buffered saline; ddH2O, double-distilled water; PFA, paraformaldehyde; H&E staining, hematoxylin-eosin staining.