High-Throughput-Compatible Differentiation of Human Pluripotent Stem Cell Lines to Kidney Organoids for Nephrotoxic Drug Screening

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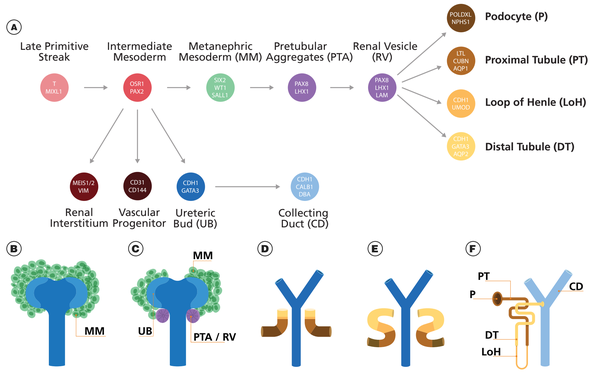
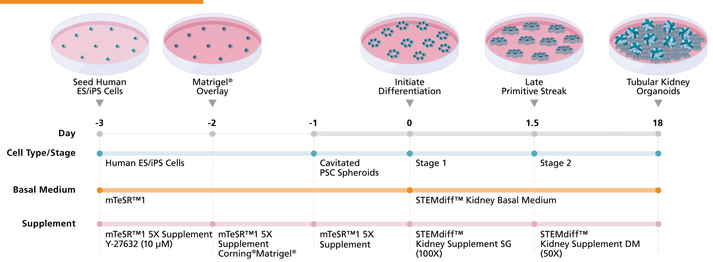
INTRODUCTION

Chronic kidney disease (CKD) represents a significant global health problem and is associated with high economic costs to our healthcare system. CKD is the condition of gradual loss of kidney function by irreversible damage to nephrons, which affects about 10% of the adult population worldwide. The ability to differentiate human embryonic stem (ES) and induced pluripotent stem (iPS) cells to functional kidney tissues provides novel tools for the development of new treatments to slow down kidney disease progression. Furthermore, the discovery of kidney organoids, which are self-organizing 3D structures containing functional renal cell types resembling some aspects of the in vivo counterpart, overcomes the limitation of insufficient modeling of cellular interactions in common monolayer culture systems. Kidney organoids offer new opportunities to model patient-specific kidney disease, study kidney development, and perform nephrotoxic compound screening. In recent years, several groups have established direct differentiation protocols by guiding human pluripotent stem cells (hPSCs) in a stepwise manner through stages of late primitive streak, intermediate mesoderm, and metanephric mesoderm to give rise to pretubular aggregates, then renal vesicles that ultimately form kidney organoids (Figure 1). However, many protocols require differentiation cultures to be dissociated into single-cell suspensions and re-aggregated during their differentiation, which may result in decreased efficiencies, lower kidney organoid yields, and higher experimental variability. To standardize the generation of kidney organoids, we developed STEMdiff™ Kidney Organoid Kit, containing a specialized serum-free medium formulation that enables highly efficient and reproducible differentiation of hPSCs to kidney organoids that model the developing nephron—composed of podocytes and proximal and distal tubules—and its associated endothelium and mesenchyme. Furthermore, we minimized cell culture manipulations with a simple two-stage differentiation system, which is compatible with high-throughput phenotypic screening in 96- and 384-well plates.



METHODS



RESULTS

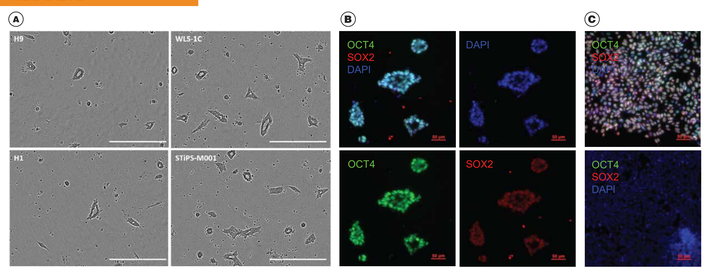


FIGURE 1. Kidney Development
(A) In mammals, nephrons are generated by sequential lineage commitment of late primitive streak cells into posterior intermediate mesoderm, metanephric mesoderm (MM), pretubular aggregates (PTA), and renal vesicles (RV) that further differentiate into podocytes (P), proximal tubules (PT), Loop-of-Henle (LoH), and distal tubules (DT). Intermediate mesoderm also gives rise to other kidney cell types, namely renal interstitium, vascular progenitor, and ureteric bud (UB). The latter forms the collecting duct (CD). Typical markers of each individual stage of development are highlighted in circles. **(B)** The metanephric mesenchyme condenses around the ureteric bud tips and forms the **(C)** pretubular aggregate followed by renal vesicle, which undergo anatomical stages of **(D)** comma-shaped body, **(E)** S-shaped body, and develops into **(F)** the functional nephron.

FIGURE 2. Overview of the Morphological Changes Over the Course of Differentiation and Two-Stage Protocol Schematic for STEMdiff™ Kidney Organoid Kit
Human ES and iPS cell lines, previously maintained in mTeSR™1, were seeded into Corning® Matrigel®-coated 96-well plates. After 24 hours, adherent cells were overlaid with an additional layer of Matrigel®, which resulted in the formation of cavitated PSC spheroids within the next 48 hours. On the following day (Day 0), differentiation of cavitated PSC spheroids was initiated by switching the medium from mTeSR™1 to STEMdiff™ Kidney Organoid Kit. During the next 18 days of differentiation, cells were directed through stages of late primitive streak, posterior intermediate mesoderm, and metanephric mesoderm to give rise to kidney organoids that are composed of endothelial cells, mesenchymal cells, podocytes, and proximal and distal tubules.

FIGURE 3. Overlay of Single-Cell Seeded hPSCs with Corning® Matrigel® Efficiently Generates Undifferentiated, Cavitated PSC Spheroids
At 24 hours post seeding of single-cell suspensions, cells were overlaid with Matrigel®, maintained for 2 additional days in mTeSR™1, and cavitated PSC spheroids were analyzed by **(A)** bright-field microscopy (scale bars = 450 µm) and **(B)** immunocytochemistry staining for co-expression of markers of the undifferentiated stem cell stage OCT4 and SOX2. Efficient and robust formation of cavitated PSC spheroids was observed across multiple human ES cell (H9, H1) and iPS cell (WLS-1C, STiPS-M001) lines, which uniformly express markers OCT4 and SOX2 of undifferentiated stem cells. **(C)** Biological controls were simultaneously stained with antibodies against OCT4 and SOX2 and analyzed by fluorescent immunocytochemistry. H9 ES cells maintained on Matrigel® cultured without an overlay retained high expression of both OCT4 and SOX2 (top image). Negative control of H9 ES cells differentiated to mid-/hindgut (bottom image).

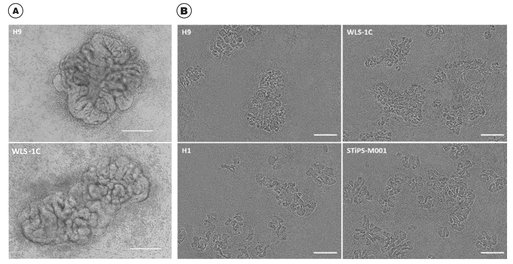


FIGURE 4. Efficient Differentiation of hPSCs into Self-Organizing Kidney Organoids
(A) Bright-field microscopy of Day 18 kidney organoids derived from H9 ES cells or WLS-1C iPS cells (scale bars = 200 µm). **(B)** Lower magnification of various ES cell (H9 or H1)-derived and iPS cell (WLS-1C or STiPS-M001)-derived kidney organoids on Day 15 of differentiation (scale bars = 450 µm). **(C)** Manual quantification of kidney organoids per well (0.32 cm^2 growth area) of a 96-well plate on Day 18 generated from multiple ES cell (H9,H1) and iPS cell (WLS-1C,STiPS-M001) lines using either STEMdiff™ Kidney Organoid Kit or homemade medium as published by Freedman et al.[1] (mean ± SD, n ≥ 2 as shown in the graph). All four cell lines tested were capable of differentiating into self-organizing kidney organoids that form convoluted tubular structures with high efficiency.

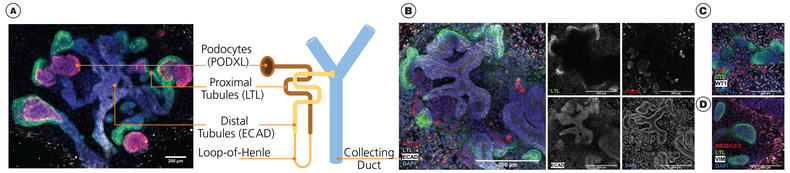


FIGURE 5. hPSC-Derived Kidney Organoids Express Key Kidney Markers
(A) Comparison of an immunofluorescence-labeled kidney organoid with a simplified schematic showing the composition of a nephron. Organoids, analyzed on Day 18, generated self-organizing kidney organoids that form convoluted tubular structures with typical nephron-like segmentation marked by expression of podocyte (podocalyxin [PODXL]), proximal (lotus tetragonolobus lectin [LTL]), and distal tubule (E-cadherin [ECAD]) markers. **(B)** Fluorescent immunocytochemistry analysis of H9 ES and STiPS-M001 iPS cells differentiated for 18 days express podocyte and proximal and distal tubule-specific markers PODXL, LTL, ECAD, **(C)** endothelial marker CD31 (platelet endothelial cell adhesion molecule), metanephric mesenchyme/podocyte marker WT1 (Wilms tumor protein 1), and **(D)** stromal markers MEIS1/2/3 (Meis homeobox 1/2/3) and VIM (vimentin). Scale bars = 200 µm.

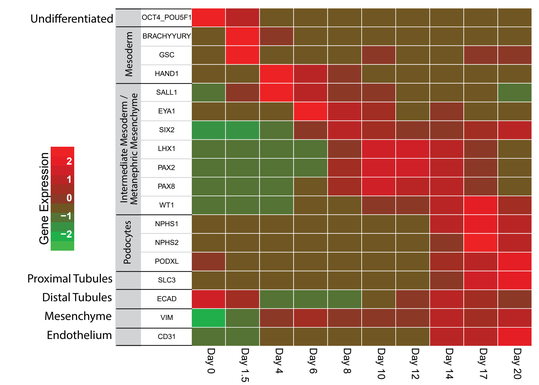


FIGURE 6. Changes in Gene Expression in Differentiation Cultures Mimic Kidney Development
Kidney organoids cultured using STEMdiff™ Kidney Organoid Kit exhibit the expected changes in gene expression as the cells differentiate and form organoids with podocytes, proximal tubules, distal tubules, and the associated mesenchyme and endothelium. Marker levels were assessed in four independent experiments by RT-qPCR, normalized to expression levels of housekeeping genes, and displayed as fold change differences from undifferentiated cells.

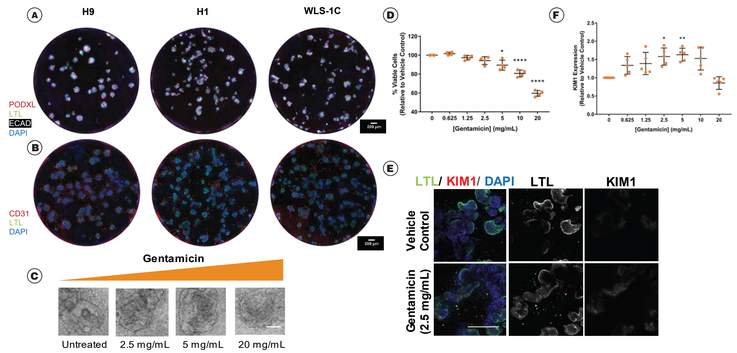


FIGURE 7. STEMdiff™ Kidney Organoid Kit is Compatible With High-Throughput Formats and Enables Screening of Nephrotoxic Drugs
(A) Whole-well imaging of H9 ES, H1 ES, and WLS-1C iPS cell-derived kidney organoids differentiated for 18 days and fluorescently labeled with a combination of PODXL, LTL, ECAD, DAPI, or **(B)** CD31, LTL, DAPI (scale bars = 200 µm. **(C)** Assessment of kidney organoid-specific cytotoxicity after gentamicin treatment, which causes damage to renal tubules as observed by bright-field microscopy (scale bar = 50 µm) and **(D)** reduced cell survival in a dose-dependent manner using a quantitative, luminescence-based viability assay. **(E)** Human PSC-derived kidney organoids express kidney injury molecule-1 (KIM-1), a specific biomarker expressed in damaged tubules, upon treatment of organoids with a sub-lethal dose of gentamicin as assessed by immunofluorescence analysis (scale bar = 200 µm) or **(F)** measured by ELISA.

Summary

- Kidney organoids generated using STEMdiff™ Kidney Organoid Kit model the developing nephron with its typical segmentation of podocytes and proximal and distal tubules, and the associated endothelium and mesenchyme
- STEMdiff™ Kidney Organoid Kit promotes efficient and reproducible differentiation across multiple ES and iPS cell lines due to its optimized formulation and rigorous quality control
- Kidney organoids were generated using a simple, two-stage differentiation with minimized culture manipulations and following an easy-to-use protocol
- Differentiation of hPSCs to kidney organoids using STEMdiff™ Kidney Organoid Kit is compatible with 96- and 384-well plates for high-throughput assays such as nephrotoxic compound screening

References
1. Freedman BS et al. (2015) Nat Commun 6: 8715.

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