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

Colleen F. Umali¹, Ryan K. Conder¹, Allen C. Eaves¹,², Sharon A. Louis¹, and Philipp M. Kramer¹

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 15% 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 study kidney physiology and 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 study kidney physiology and disease progression.

METHODS

RESULTS

FIGURE 1. Kidney Development

(A) Human ES and iPS cells were overlaid on a scaffold of Matrigel®, which resulted in the formation of 3D PSC spheroids within the next 48 hours. By Day 2, differentiation of spheroid aggregates was initiated by switching the medium from mTeSR™1 5X Supplement to STEMdiff™ Kidney Organoid Kit. During the next 16 days of differentiation, cells were cultured through stages of late primitive streak, posterior intermediate mesoderm, and metanephric mesoderm to give rise to posterior intermediate mesoderm, and metanephric mesoderm aggregates that ultimately form kidney organoids (Figure 1). However, many protocols require complex, time-consuming culture manipulations and following an easy-to-use protocol.

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 PSCs were previously maintained in mTeSR™1 5X Supplement and were seeded into Corning Matrigel-coated 96-well plates. After 24 hours, sufficient cells were overlaid with an addition of Matrigel®, which resulted in the formation of 3D PSC spheroids within the next 48 hours. By Day 2, differentiation of spheroid aggregates was initiated by switching the medium from mTeSR™1 5X Supplement to STEMdiff™ Kidney Organoid Kit. During the next 16 days of differentiation, cells were cultured through stages of late primitive streak, posterior intermediate mesoderm, and metanephric mesoderm to give rise to posterior intermediate mesoderm, and metanephric mesoderm aggregates that ultimately form kidney organoids (Figure 1). However, many protocols require complex, time-consuming culture manipulations and following an easy-to-use protocol.

FIGURE 3. Overview of Single-Cell Seeded PSCs with Corning Matrigel® Efficiently Generates Undifferentiated, Cardiac and Neural Spheres

At 24 hours post-seeding of single-cell suspensions, cells were overlaid on Matrigel®. The differentiation protocol was extended for 2 additional days in mTeSR™1 5X Supplement, and cultured PSC spheroids were analyzed by (A) bright-field microscopy. Scale bars = 400 μm and (B) immunocytochemical staining for co-expression of markers of the undifferentiated stem cell stage OCT4 and SOX2. Efficient and uniform formation of spheroid PSCs was observed across multiple human ES cell (H1 and H9) and PSC (H1 or H9) lineages, which uniformly expressed markers OCT4 and SOX2 of undifferentiated stem cells (A). Biological controls were performed using either adherent or non-adherent PSC cultures. Neither OCT4 nor SOX2 expression was assessed by fluorescent immunocytochemistry (B). Immunostaining for OCT4 and SOX2 was analyzed by fluorescent immunocytochemistry. H9 ES cells were overlaid on Matrigel® cultured without an overlay, in agreement with the expected high expression of both OCT4 and SOX2 (top image). Negative control of H9 ES cells cultured without Matrigel® (bottom image).

FIGURE 4. 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 kidneys differentiate and form organoids with podocytes, proximal tubules, distal tubules, and the associated endothelium and mesenchyme. The markers are 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 5. HPS-C-derived Kidney Organoids Express Key Kidney Markers

Kidney organoids were generated using a simple, two-stage differentiation with minimized culture manipulations and following an easy-to-use protocol using either STEMdiff™ Kidney Organoid Kit or homemade STEMdiff™ Kidney Basal Medium and kidney Supplement DM. Kidney organoids 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 study kidney physiology and disease progression.

FIGURE 6. Efficient Differentiation of PSCs into Self-Organizing Kidney Organoids

(A) Bright-field microscopy of Day 16 kidney organoids derived from H9 ES cells or WLS-1C iPS cells (scale bars = 400 μm). (B) Immunofluorescence staining for podocyte marker WT1 (Wilms tumor protein 1), and (C) mesenchymal/endothelial marker VWF (von Willebrand factor) with DAPI and fluorescently labeled with a combination of PODXL, LTL, ECAD, or DAPI, or podocyte marker WT1 (Wilms tumor protein 1), and (C) mesenchymal/endothelial marker VWF (von Willebrand factor) with DAPI and fluorescently labeled with a combination of PODXL, LTL, ECAD, or DAPI. Comparison of an immunofluorescence-labeled kidney organoid with a representative secondary antibody control. kid 18 days differentiated with a typical nephron segmentation. Kidney organoids, analyzed on Day 18, generated self-organizing kidney organoids that form convoluted tubular structures with typical nephron segmentation. Kidney organoids cultured using STEMdiff™ Kidney Organoid Kit exhibit the expected changes in gene expression as the kidneys differentiate and form organoids with podocytes, proximal tubules, distal tubules, and the associated endothelium and mesenchyme.

FIGURE 7. STEMdiff™ Kidney Organoid Kit is Compatible With High-Throughput Formats and Enables Screening of Nephrotoxic Drugs

(A) Whole-cell imaging of H9 ES, H1 ES, and WLS-1C iPS cells differentiated for 18 days were analyzed with a confocal imaging system. Kidney organoids were assessed by high-throughput imaging microscopy (scale bar = 50 μm) and the associated endothelium and mesenchyme. The markers are assessed in four independent experiments by RT-qPCR, normalized to expression levels of housekeeping genes, and displayed as fold change differences from undifferentiated cells.

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. The markers are assessed in four independent experiments by RT-qPCR, normalized to expression levels of housekeeping genes, and displayed as fold change differences from undifferentiated cells.