Results: Differentiation of iPSC-derived endothelial cells and endothelial-specific function such as permeability, development of glycocalyx, and permeability are significantly improved by microfluidity conditions. In the presence of macrophages the number of endothelial cells is significantly increased. Furthermore, co-culture with macrophages induced luminal formation and capillary capillary growth. Formed capillary showed normal growth to the glomeruli-like nephrin-positive vascular clusters and tend to interconnect developing neighboring organoids. The analysis of endothelial cells transcriptome shows advanced differentiation pattern in the presence of macrophages in comparison to the organoids in monoculture.

Conclusions: In this study, we firstly demonstrate that co-culturing developing iPSC-kidney organoids with human iPSC-macrophages promotes endothelial differentiation and induces capillary formation. Macrophages may directly or indirectly contribute to the early vascularization in organoids and can improve organoid-derived renal tissue generation.

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FR-PO360
Kidney Decellularized Extracellular Matrix Enhanced the Vascularization and Maturation of Human Kidney Organoids
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Background: Kidney organoids derived from human pluripotent stem cells have extensive potential for disease modelling and regenerative medicine. However, the limited vascularization and immaturity of kidney organoids have been still remained to overcome. In kidney development, kidney ECM regulate mesenchymal condensation, nephron specification, terminal differentiation of renal tubules, and glomerular basement membrane assembly. Kidney decellularized ECM (dECM) hydrogels contain ECM proteins to provide a microenvironment similar to that of a normal kidney. Our study highlights that kidney dECM hydrogels could be used to more accurately culture kidney organoids.

Methods: Porcine kidneys were decellularized to prepare kidney decellularized extracellular matrix hydrogels. Kidney organoids were differentiated using kidney dECM, and VEGF for enhancing the vascular network and SB-431542 for enhancing podocyte differentiation towards the glomerulus-like nephron-positive vascular clusters (A(GLA) mutant ESCs in which the GLA gene was knocked out were generated using the CRISPR/Cas9). To recapitulate Fabry Nephropathy with Vasculopathy, GLA-mutant human iPSCs were differentiated into kidney organoids using kidney dECM. We transplanted kidney organoids derived from differentiated iPSCs with kidney dECM beneath the kidney capsule of immunodeficient NOD-SCID mice for engraftment.

Results: The vascularization was extensively increased in the kidney organoids generated by using kidney dECM. Single-cell transcriptomics revealed that the vascularized kidney organoids cultured using the kidney dECM had more mature patterns of glomerular development and higher similarity to human kidney than those cultured without the kidney dECM. Differentiation of GLA knock-out hPSC generated using CRISPR/Cas9 into kidney organoids by the culture method using kidney dECM efficiently recapitulated Fabry nephropathy with vasculopathy. Transplantation of kidney organoids with kidney dECM into mice of NOD-SCID mice accelerated the recruitment of endothelial cells from the host kidney and maintained vascular integrity with the more organized slit diaphragm-like structures than those without kidney dECM.

Conclusions: Our study suggests that kidney dECM methodology for inducing extensive vascularization and maturation of kidney organoids can be applied to studies for kidney development, disease modeling, and regenerative medicine.

FR-PO361
Developing Methods to Improve Vascularization of Nephron Progenitor Cell Grafts Beneath the Kidney Capsule Through the Use of Pro-Angiogenic Uniformly Porous Templated Hydrogel Scaffolds
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Background: When human kidneys are acutely injured or damaged by underlying chronic conditions, the nephrons are unable to sufficiently repair themselves and will progressively deteriorate to the point of end stage kidney disease. The fairly recent development of stem cell-derived kidney organoids has raised the possibility of achieving regenerative medicine-based therapeutic to restore renal function. However, studies in vivo have not yet achieved clinically significant integration of implanted cells with the host kidney. Here we examine the effects of implanting nephron progenitor cells (NPCs) as well as pro-angiogenic uniform porous templated hydrogel scaffolds beneath the kidney capsule as potential methods to achieve highly vascularized grafts.

Methods: Human induced pluripotent stem cell-derived NPCs, differentiated kidney organoids, and hydrogel scaffolds were implanted beneath the kidney capsules of 8-10-week-old NOD-SCID mice. Cellular material was collected by manually scraping cells from adherent cultures in a 24-well plate and aggregated through centrifugation. Kidneys were excised after 3-weeks and analyzed through cryosection immunofluorescent staining for CD31 and VE-Cadherin. Characterization of implanted NPCs with kidney dECM supported differentiation and led to the development of renal structures that include podocytes, and proximal and distal tubules. The developing podocytes interact with host vasculature to form chimeric glomerular structures lined by parietal epithelial cells. Additionally, implanted porous scaffolds allow for ubiquitous cellular infiltration and vascularization while reducing the fibrotic host response compared to similar non-porous implants.

Conclusions: Together, the effects of these experiments have demonstrated the potential for each of these two technologies to improve kidney regeneration therapies. Next steps will be to optimize cell-seeding methodology and implant combined NPC-seeded scaffolds beneath the kidney capsule to explore potential synergistic effects.

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FR-PO362
Utilizing a Patient-Specific iPSC Platform for the Study of Rare Genetic Kidney and Vascular Diseases
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Background: The increasing use of induced pluripotent stem cells (iPSCs) to model human genetic diseases in vitro has allowed researchers to better understand the pathology of countless disorders, and subsequently develop better treatments for patients. Currently in our lab we use blood-outgrowth endothelial cells (BOECs) to study endothelial cell (EC) dysfunction in kidney diseases and thrombotic microangiopathies. However, isolating patient BOECs is often challenging, as it requires a significant sample of fresh blood which is not always readily available. As such, we are working to utilize a platform that uses patient skin fibroblasts to create patient-specific iPSCs, that can then be differentiated into ECs which capture the genetic conditions of patients.

Methods: Patient skin fibroblast-derived iPSCs and healthy control iPSCs were created using a commercially available Sendai virus CytoTune-iPS 2.0 reprogramming kit (Thermo Fisher). These iPSCs were then differentiated into ECs using a directed differentiation protocol in factor-defined media. Following differentiation, CD31 (PECAM1) positive cells were sorted using fluorescence-activated cell sorting (FACS), to isolate a pure population. Isolated ECs were then characterized using immunofluorescence (IF) staining for CD31 and VE-Cadherin, as well as a tubule formation assay.

Results: We were able to successfully create the iPSCs and differentiate them into ECs that express the appropriate markers (CD31 and VE-Cadherin) and are able to successfully form robust capillary-like tubes over a 48 hour period.

Conclusions: Our iPSC differentiation protocol provides a verified and valuable tool for the study of genetic kidney disorders involving endothelial cells when BOEC isolation is not possible.

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