Exploring kidney organoid vascularization

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CHAPTER 1

Introduction and outline of this thesis

Chronic kidney disease (CKD) is highly prevalent worldwide, affecting more than 10% of the general population[1]. It significantly increases the risk of cardiovascular and all-cause mortality[1-4] and carries a substantial economic burden that rises with disease severity[5]. Treatment options to prevent or slow progression of CKD are limited and a proportion of patients develops kidney failure. Once kidney failure occurs, patients can be treated with conservative management, dialysis, or kidney transplantation. Conservative management consists of the management of symptoms of kidney failure and preservation of residual kidney function for as long as possible, without dialysis or transplantation. Dialysis, in the form of hemodialysis or peritoneal dialysis, can replace around 10-15% of kidney function. These are strenuous treatments, with great impact on daily life and high morbidity and mortality. Kidney transplantation is currently the treatment option with the best outcomes. However, the shortage of donor organs leads to waiting lists that in the Netherlands are lengthening every year[6]. Also, some patients are too frail to undergo transplant surgery and the lifelong immunosuppressive treatment that is required after transplantation. There is therefore an urgent need for new methods to treat patients with CKD.

Kidney organoids

Kidney organoids generated from human stem cells hold great promise in this respect. In 2007, it was discovered that adult cells can be reprogrammed to stem cells[7]. These human induced pluripotent stem cells (hiPSCs) can be generated from healthy or diseased individuals, and possess the same properties as embryonic stem cells. They can proliferate indefinitely as stem cells and have the potential to differentiate to any specialized cell type in the human body. The significance of this discovery was immediately recognized by researchers, and since then protocols have been developed for the differentiation of hiPSCs to all kinds of cell types, including kidney organoids[8-12].

Protocols for the generation of kidney organoids from hiPSCs are based on embryonic kidney development. In embryology, kidneys develop from 2 progenitor populations: the metanephric mesenchyme, which forms the nephron, and the ureteric bud, which gives rise to the collecting duct and ureter. Both progenitor populations are derived from the intermediate mesoderm, which develops from primitive streak cells that migrate anteriorly. Kidney organoid differentiation protocols attempt to mimic this process in vitro: hiPSCs are treated with CHIR to mimic Wnt signaling inducing differentiation to primitive streak, followed by FGF9 to induce intermediate mesoderm. An additional CHIR pulse as well as interaction between the different developing cells in the organoids leads to further differentiation. Interestingly, the relatively straightforward protocols for the generation of kidney organoids yield highly complex nephron structures, consisting of glomerular,

proximal tubular, and distal tubular cells that are surrounded by stromal and endothelial cells (ECs). Organoids in their current form have already been used to screen for drug toxicity and model certain early onset kidney diseases[13-18], which will hopefully in the future reduce the need for laboratory animals. However, their impact would be much greater if they could be used for transplantation as auxiliary kidney tissue or even to fully replace kidney function.

Several important limitations currently hinder kidney organoids from achieving their full potential: Kidney organoids contain off-target cell populations causing safety concerns[19,20], they are far too small to generate sufficient kidney function to keep patients off dialysis, and 3D organization with a urine drainage system is lacking. Another major concern is that kidney organoids lack a functional vasculature and are (ultra)structurally, functionally and metabolically immature.

Vascularization of human kidneys versus kidney organoids

Kidneys are highly vascularized organs, receiving 20-25% of cardiac output. Blood flow is indispensable for kidney function, allowing for oxygenation of renal cells, glomerular filtration, blood pressure regulation, tubular reabsorption and secretion. In addition to blood flow, the ECs lining the renal vasculature play a crucial role in kidney development and maintenance. Renal ECs are phenotypically heterogeneous to support the specific functions of each region of the kidney[21,22]. Glomerular ECs are the most well-known example in this respect. These highly fenestrated cells, together with podocyte foot processes and the glomerular basement membrane (GBM), form the glomerular filtration barrier that allows for the continuous filtration of large volumes of blood. The development of this intricate structure requires interaction between glomerular podocytes, mesangial cells and ECs. In embryology, podocyte precursors attract ECs by producing vascular endothelial growth factor A (VEGF-A), which start to invade nephron structures in the S-shaped body stage of development. It has been shown in mouse models that a reduction of podocyte-derived VEGF-A leads to severe damage to ECs, with a complete absence of VEGF-A resulting in unvascularized glomeruli and perinatal death[23]. In turn, podocytes lacking interaction with ECs fail to mature, displaying effacement of foot processes and absence of slit diaphragms[23].

Kidney organoids cultured in vitro do contain ECs, but these are mainly located at the periphery of the organoid and fail to invade the clusters of podocytes that form the glomerular structure (Fig. 1). The interaction required for proper development of both

cell types as well as the glomerular filtration barrier consequently does not occur and the organoid glomeruli and nephrons remain immature. This poses important restrictions to the use of kidney organoids for modeling glomerular diseases as well as for regenerative medicine.

Strategies to enhance vascularization and maturation of kidney organoids

Transplantation of kidney organoids under the kidney capsule or subcutaneously in mice has been shown to induce vascularization and enhance maturation[24,25]. These transplanted organoids become functionally perfused by blood vessels that are lined mainly by host derived ECs and have even been shown to be capable of glomerular sieving[26]. The disadvantage of this model is that it is labour intensive, not allowing for high throughput experiments. Also, the factors responsible for inducing vascularization upon transplantation remain unknown. It has been hypothesized that exposure of ECs to flow, hypoxia, growth factors and the presence of mature ECs from the host might play a role.

Attempts to vascularize kidney organoids in vitro by mimicking these condition through culture under laminar flow, in a hypoxic environment, or after addition of VEGF-A to the culture medium have been only partially successful[27-29]. These strategies mainly induced an increase in the number of ECs but no or at most sporadic invasion of glomerular structures by ECs. Functional vascularization of in vitro kidney organoids by perfusable endogenous ECs therefore remains elusive.

Analyzing kidney organoids at a single cell level

For the development of new strategies to enhance kidney organoid vascularization and maturation, detailed analysis of the organoids at a molecular level can be of great value. Unfortunately, the interpretation of kidney organoid gene expression data from quantitative PCR or bulk RNA sequencing analysis is challenging. These techniques provide average gene expression levels for organoids as a whole, but do not inform about cell type specific transcriptomes. This does not do justice to the complexity of kidney organoids, as differences in gene expression between the many different cell types remain undetected. The development of single cell RNA sequencing (scRNAseq) analysis has overcome this problem. It enables the measurement of transcriptome profiles for individual cells at great scale and high resolution. This provides the possibility to distinguish cell populations, identify possible target genes for differentiation, maturation or disease modeling, and analyse potential interactions between cell populations. In this manner, it could provide important clues to enhance kidney organoid vascularization and maturation.

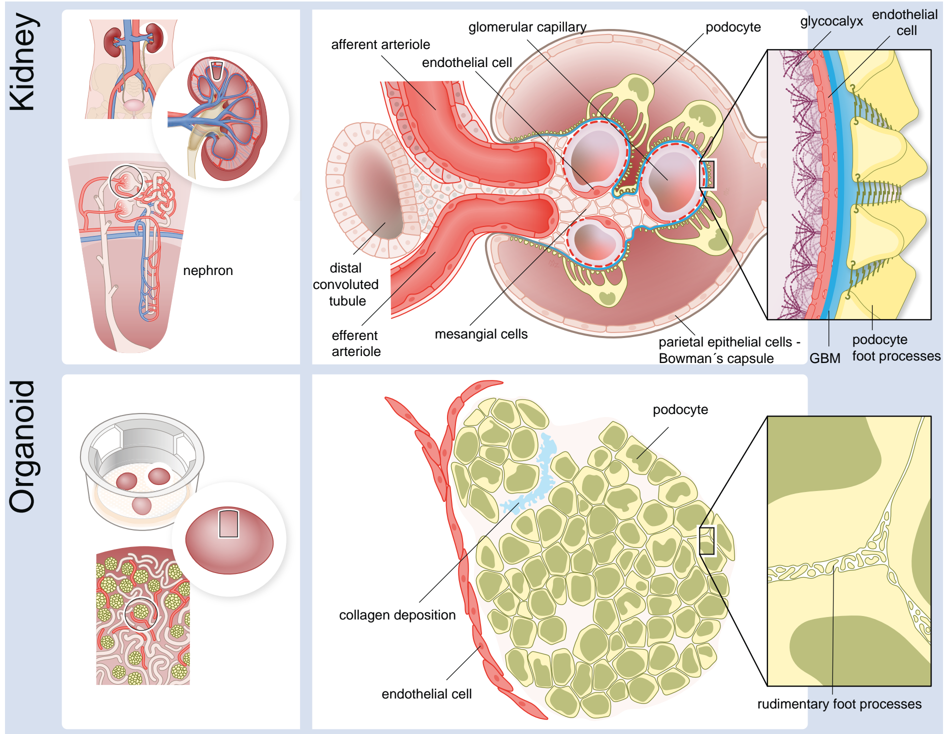

Figure 1: glomerular structures in the adult kidney (top) and kidney organoid (bottom). The glomerulus in the adult kidney consists of a network of capillaries, lined with fenestrated endothelial cells, that are supported by mesangial cells and surrounded by podocytes. Blood enters the glomerulus through the afferent arteriole. Filtration occurs through the glomerular filtration barrier, the filtrate enters Bowman's space and flows into the proximal tubule. Blood exits the glomerulus through the efferent arteriole. The glomerular filtration barrier (zoom of boxed area) consists of fenestrated endothelial cells, the glomerular basement membrane and podocyte foot processes.
Kidney organoids contain glomerular structures, tubular structures and endothelial cells. Glomerular structures in kidney organoids consist of clusters of podocytes that in some areas deposit unorganized collagen and are surrounded with but not invaded by endothelial cells. The podocytes display rudimentary foot processes, but a glomerular filtration barrier does not form.

General outline of this thesis

The aim of this thesis is to explore the process of kidney organoid vascularization and its effect on organoid development and maturation.

In **Chapter 2**, methods for the generation of kidney organoids from human iPSCs and their limitations are discussed, with a focus on vascularization. Although currently available protocols yield organoids containing nephron-like structures, the lack of a functional vasculature and consequent immaturity of the organoids limits their applicability. Existing strategies to enhance vascularization include transplantation in mice. However, this is labour intensive and unsuitable for studying large numbers of organoids.

To improve organoid vascularization, we initially attempted in vitro co-culture with ECs. In **Chapter 3,** we discuss the different strategies we applied, including exposure to flow and modifications of the extracellular matrix. Despite adaptations to the differentiation protocol and culture conditions it seems challenging to induce functional vascularization.

We therefore decided to take a step back, and study the process of organoid vascularization in more detail. For this purpose, we developed a method for intracoelomic transplantation of kidney organoids in chicken embryos. In **Chapter 4**, we show that transplanted organoids are functionally vascularized within 8 days, leading to perfusion of organoid glomeruli. Compared to untransplanted controls, transplanted organoids display enhanced maturation at a transcriptional as well as ultrastructural level. The interaction between ECs and podocytes in transplanted organoids enables the development of a rudimentary GBM. The detailed protocol for intracoelomic transplantation of kidney organoids in chicken embryos, followed by injection of fluorescently labelled lectin to visualize perfused blood vessels, can be found in **Chapter 5**.

Considering the phenotypic heterogeneity of ECs in the adult kidney, in **Chapter 6** we next evaluate the characteristics of organoid ECs in untransplanted and transplanted organoids. Human ECs were isolated from transplanted organoids and untransplanted controls and analysed using single cell RNA sequencing (scRNAseq). We show that organoid ECs are heterogeneous and that transplantation sustains EC proliferation and induces a major shift from venous to arterial ECs that resemble fetal kidney ECs. In addition, we identify possible targets to enhance vascularization.

Chapter 7 provides a discussion of the findings presented in this thesis and the future possibilities of kidney organoids for research and regenerative medicine purposes.

References

1 Kovesdy, C. P. Epidemiology of chronic kidney disease: an update 2022. Kidney Int Suppl (2011) ***12***, 7-11 (2022). https://doi.org:10.1016/j.kisu.2021.11.003

2 Matsushita, K. et al. Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. Lancet ***375***, 2073-2081 (2010). https://doi.org:10.1016/S0140-6736(10)60674-5

3 Go, A. S., Chertow, G. M., Fan, D., McCulloch, C. E. & Hsu, C. Y. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. N Engl J Med ***351***, 1296-1305 (2004). https://doi.org:10.1056/NEJMoa041031

4 van der Velde, M. et al. Lower estimated glomerular filtration rate and higher albuminuria are associated with all-cause and cardiovascular mortality. A collaborative meta-analysis of high-risk population cohorts. Kidney Int ***79***, 1341-1352 (2011). https://doi.org:10.1038/ki.2010.536

5 Jha, V. et al. Global Economic Burden Associated with Chronic Kidney Disease: A Pragmatic Review of Medical Costs for the Inside CKD Research Programme. Adv Ther ***40***, 4405-4420 (2023). https://doi.org:10.1007/s12325-023-02608-9

6 Nederlandse Transplantatie Stichting, Cijferoverzicht 2021. (2021).

7 Takahashi, K. et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell ***131***, 861-872 (2007). https://doi.org:10.1016/j.cell.2007.11.019

8 Taguchi, A. et al. Redefining the in vivo origin of metanephric nephron progenitors enables generation of complex kidney structures from pluripotent stem cells. Cell Stem Cell ***14***, 53-67 (2014). https://doi.org:10.1016/j.stem.2013.11.010

9 Morizane, R. et al. Nephron organoids derived from human pluripotent stem cells model kidney development and injury. Nat Biotechnol ***33***, 1193-1200 (2015). https://doi.org:10.1038/nbt.3392

10 Takasato, M. et al. Kidney organoids from human iPS cells contain multiple lineages and model human nephrogenesis. Nature ***526***, 564-568 (2015). https://doi.org:10.1038/nature15695

11 Freedman, B. S. et al. Modelling kidney disease with CRISPR-mutant kidney organoids derived from human pluripotent epiblast spheroids. Nat Commun ***6***, 8715 (2015). https://doi.org:10.1038/ncomms9715

12 Taguchi, A. & Nishinakamura, R. Higher-Order Kidney Organogenesis from Pluripotent Stem Cells. Cell Stem Cell (2017). https://doi.org:10.1016/j.stem.2017.10.011

13 Tanigawa, S. et al. Organoids from Nephrotic Disease-Derived iPSCs Identify Impaired NEPHRIN Localization and Slit Diaphragm Formation in Kidney Podocytes. Stem Cell Reports (2018). https://doi.org:10.1016/j.stemcr.2018.08.003

14 Forbes, T. A. et al. Patient-iPSC-Derived Kidney Organoids Show Functional Validation of a Ciliopathic Renal Phenotype and Reveal Underlying Pathogenetic Mechanisms. Am J Hum Genet ***102***, 816-831 (2018). https://doi.org:10.1016/j.ajhg.2018.03.014

15 Hale, L. J. et al. 3D organoid-derived human glomeruli for personalised podocyte disease modelling and drug screening. Nat Commun ***9***, 5167 (2018). https://doi.org:10.1038/s41467-018-07594-z

16 Cruz, N. M. et al. Organoid cystogenesis reveals a critical role of microenvironment in human polycystic kidney disease. Nat Mater (2017). https://doi.org:10.1038/nmat4994

17 Dorison, A. et al. Kidney Organoids Generated Using an Allelic Series of NPHS2 Point Variants Reveal Distinct Intracellular Podocin Mistrafficking. J Am Soc Nephrol (2022). https://doi.org:10.1681/ASN.2022060707

18 Vanslambrouck, J. M. et al. Enhanced metanephric specification to functional proximal tubule enables toxicity screening and infectious disease modelling in kidney organoids. Nat Commun ***13***, 5943 (2022). https://doi.org:10.1038/s41467-022-33623-z

19 Wu, H. et al. Comparative Analysis and Refinement of Human PSC-Derived Kidney Organoid Differentiation with Single-Cell Transcriptomics. Cell Stem Cell (2018). https://doi.org:10.1016/j.stem.2018.10.010

20 Phipson, B. et al. Evaluation of variability in human kidney organoids. Nat Methods ***16***, 79-87 (2019). https://doi.org:10.1038/s41592-018-0253-2

21 Dumas, S. J. Single-Cell RNA Sequencing Reveals Renal Endothelium Heterogeneity and Metabolic Adaptation to Water Deprivation. JASN (2019).

22 Dumas, S. J. et al. Phenotypic diversity and metabolic specialization of renal endothelial cells. Nat Rev Nephrol ***17***, 441-464 (2021). https://doi.org:10.1038/s41581-021-00411-9

23 Eremina, V. et al. Glomerular-specific alterations of VEGF-A expression lead to distinct congenital and acquired renal diseases. J Clin Invest ***111***, 707-716 (2003). https://doi.org:10.1172/JCI17423

24 van den Berg, C. W. et al. Renal Subcapsular Transplantation of PSC-Derived Kidney Organoids Induces Neo-vasculogenesis and Significant Glomerular and Tubular Maturation In Vivo. Stem Cell Reports ***10***, 751-765 (2018). https://doi.org:10.1016/j.stemcr.2018.01.041

25 Bantounas, I. et al. Generation of Functioning Nephrons by Implanting Human Pluripotent Stem Cell-Derived Kidney Progenitors. Stem Cell Reports ***10***, 766-779 (2018). https://doi.org:10.1016/j.stemcr.2018.01.008

26 van den Berg, C. W., Koudijs, A., Ritsma, L. & Rabelink, T. J. In Vivo Assessment of Size-Selective Glomerular Sieving in Transplanted Human Induced Pluripotent Stem Cell-Derived Kidney Organoids. J Am Soc Nephrol ***31***, 921-929 (2020). https://doi.org:10.1681/ASN.2019060573

27 Homan, K. A. et al. Flow-enhanced vascularization and maturation of kidney organoids in vitro. Nat Methods ***16***, 255-262 (2019). https://doi.org:10.1038/s41592-019-0325-y

28 Menéndez, A. B. C. et al. Creating a kidney organoid-vasculature interaction model using a novel organ-on-chip system. Sci Rep-Uk ***12*** (2022). https://doi.org:ARTN 20699 10.1038/s41598-022-24945-5

29 Schumacher, A. et al. Enhanced Microvasculature Formation and Patterning in iPSC-Derived Kidney Organoids Cultured in Physiological Hypoxia. Front Bioeng Biotechnol ***10***, 860138 (2022). https://doi.org:10.3389/fbioe.2022.860138