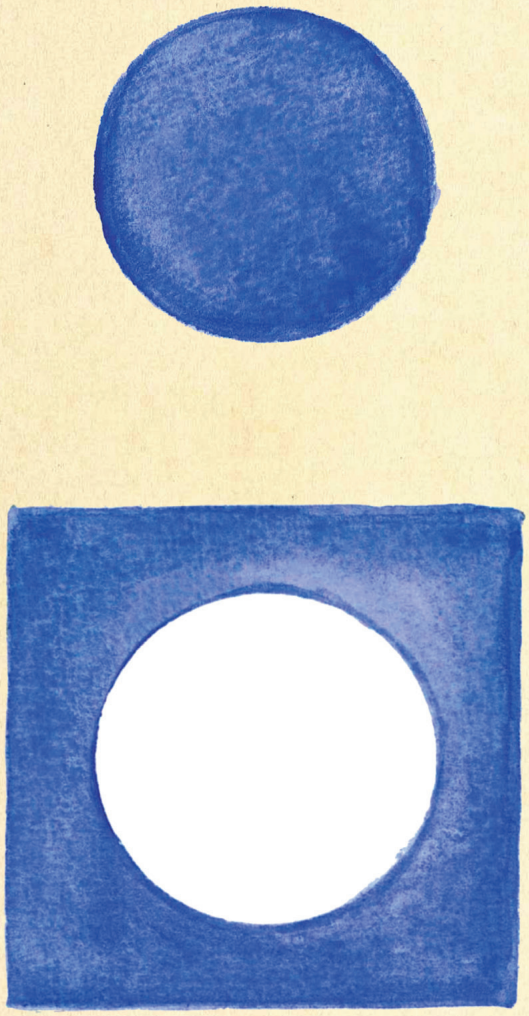
From biofabrication to multi-organ platforms: engineering the human heart-kidney axis in vitro

Gabbin, B.



Chapter 6

General discussion

From simplistic to complex systems for modeling multi-organ interactions *in vitro*: the heart-kidney axis as an example

Human induced pluripotent stem cell (hiPSC)-derived organoids and organ-on-chip (OoC) platforms are progressively transforming our ability to model *in vitro* human physiology and disease. In this thesis, we have developed and validated an advanced *in vitro* system to study the cardiorenal axis, investigating the extent to which we can model the intricate relationship between the heart and kidneys. This thesis demonstrated that integrating hiPSC-derived cardiac microtissues (cMTs) and kidney organoids (kOs) in controlled static and dynamic platforms not only recapitulates fundamental aspects of organ-specific function, but also enables the capture of inter-organ signaling mechanisms relevant to pathologies, such as the cardiorenal syndrome (CRS), which is reviewed in depth in Chapter 2.

Traditionally, animal models have served as the pillars of biomedical research. However, interspecies differences in renal and cardiovascular genetics, anatomy, physiology and immune responses appear to limit their predictive value for human applications. Recent advancements in *in vitro* disease modeling mark a shift in our field as, through hiPSCs, human-relevant systems that have the potential to become the gold standard for translational studies are being developed. Moreover, to aid reproducibility and standardization of protocols, centralized facilities that facilitate the availability of well-characterized hiPSC lines, such as the iPSC Hotel in the LUMC, enable the generation of research grade hiPSC cell lines from patients and healthy control individuals which can be used for robust organoid generation[1]. Our work reinforces the increasing relevance and maturity of human-based *in*

vitro models. This aligns with national efforts such as the Dutch Initiative for Animal-Free Innovation ("Transitie Proefdiervrije Innovatie" (TPI)) and Young TPI, which advocate the ethical and scientific advantages of replacing animal models in research[2,3].

Batch-to-batch variability of differentiated cells and hiPSC donors as well as the lack of standardization in differentiation protocols of hiPSC-derived models may introduce heterogeneity that can bias interpretation of results or limit reproducibility across laboratories. International consortia are working to establish standards that promote reproducibility, scalability, and regulatory acceptance. For cardiac models, initiatives like CiPA[4,5], the Japan iPS Cardiac Safety Assessment (JiCSA)[6], the Consortium for Safety Assessment using Human iPS cells (CSAHi)[7], and InPulse CRACK-IT[8] are (or have been) coordinating the evaluation of cardiac differentiation protocols, functional assays, and toxicity screening pipelines. For kidney organoids, the NIH-funded Kidney Precision Medicine Project (KPMP)[9] is defining protocols for organoid generation and validation using multi-omics and structural benchmarking. The European Kidney Health Alliance (EKHA)[10], although more policy and awareness-oriented, collaborates with scientific partners to promote the clinical translation and standardization of new kidney research tools, including organoids. In the Netherlands, the hDMT[11] consortium also supports standardization efforts of hiPSC models, including integration into OoC platforms. These efforts are further empowered by regulatory movements such as the FDA Modernization Act 2.0 from 2022 and the more recent 3.0, which explicitly support the use of validated non-animal, human-relevant models (such as hiPSCs, organoids and OoC) in safety and (drug) efficacy testing[12,13].

The establishment of dual-organ models, especially when enhanced by emerging technologies such as microfluidics and 3D bioprinting, positions these systems not as supplements, but as potential replacements for *in vivo* models in preclinical research and therapy development.

Relevance, limitations and outlook of our cardiac and renal *in vitro* models

Human iPSC-derived cMTs and kOs have emerged as powerful platforms for modeling organ-specific and inter-organ physiology *in vitro*. These models recapitulate key structural and functional features of the heart, such as spontaneous contraction, electrophysiological responsiveness, and of the kidney, such as nephron-like architecture, enabling the study of disease mechanisms, drug toxicity, and inter-organ crosstalk relevant to syndromes like CRS[14–17]. Moreover, their capacity for patient-specific genetic context modeling with hiPSC lines and various options for engineering genetic variants and reporters offered by gene-editing technologies makes them well-suited for personalized medicine applications.

Despite their promise, both systems face significant limitations which impact their reproducibility and predictive values. While valuable for modeling aspects of human cardiac physiology, cMTs cannot reach a fully mature adult state and do not recapitulate all structural and functional characteristics of the adult human heart[8]. Approaches such as mechanical or electrical stimulation and matrix engineering are being explored to improve cardiac maturation[14,18,19]. kOs, similarly, lack *in vitro* vascularization and exhibit limited nephron maturation and structural heterogeneity due to off-target differen-

tiation[20–22]. Strategies like progenitor enrichment, extracellular matrix bioengineering, and bioprinting (as shown in Chapter 4) are enhancing spatial fidelity and structural consistency[23–25].

Efforts to improve physiological relevance also include integration with dynamic platforms such as OoC systems (Chapter 3), which support perfusion, nutrient exchange, and inter-organ communication, particularly important for modeling diseases like CRS that involve bidirectional signaling[26,27].

Together, these advances indicate that hiPSC-derived cardiac and kidney models are on a trajectory toward their adoption in disease modeling, drug development, and personalized medicine. Their convergence in multi-organ platforms opens promising opportunities to study complex systemic diseases like CRS with unprecedented precision.

In this context, our work presented in Chapters 3–5 contributes key innovations to this field, particularly through the development of an integrated cardiorenal co-culture model and bioprinting strategies that improve reproducibility and accessibility.

Assembloids in multi-organ disease modeling

While OoC systems use engineered physical connections between tissues, an alternative approach has emerged through the development of *assembloids*: self-organizing structures composed of multiple organoid types or integrated with primary tissue explants. Assembloids offer the potential to recapitulate spatial and functional complexity without external infrastructure, relying instead on intrinsic developmental cues[28,29].

These models have gained traction particularly in neuroscience, but their

relevance is expanding to multi-organ studies[30–32]. However, reproducibility remains a significant concern, as does the lack of vasculature, which limits physiological accuracy. Without perfusion or vascularization, diffusion-based nutrient delivery is inadequate, especially for larger or highly metabolically active tissues[33].

When studying the cardiorenal axis, assembloids may complement but not yet replace OoC systems. The communication between heart and kidney occurs not because of their proximity, but through the cardiovascular system and an intricate network of complex biological mechanisms[34,35]. The precision and control provided by microfluidic perfusion are critical when studying dynamic processes like CRS, where flow, pressure, and bidirectional signaling are key drivers of pathology. Future advances may integrate vascularized assembloids with perfused chips, combining the strengths of both systems.

Bioprinting and the growing accessibility to complex model fabrication

While dynamic platforms like OoCs improve physiological relevance, the reliability, reproducibility and scalability of organoid generation remain a technical bottleneck due to the manual, labor-intensive generation of organoids. Chapter 4 of this thesis presents how 3D bioprinting offers a strong solution to these challenges for kO engineering. By implementing a cost-effective, open-source, desktop extrusion-based bioprinter, we enabled rapid, reproducible, and medium-to-high-throughput generation of kOs, which maintained structural and functional fidelity while minimizing operator-induced variability.

This approach represents more than a technical improvement, as by leveraging commercially available hardware and open-source customization, we lowered the barrier to entry for advanced tissue bioengineering, allowing laboratories with limited resources to fabricate high-quality organoids. This aligns with the principles of open science and promotes access to state-of-the-art platforms across research environments.

Beyond reproducibility and scalability, advanced bioprinting enables fine control over cell number, volume, and spatial arrangement. In our work, we demonstrated successful downscaling of organoid size without compromising nephron architecture or function, paving the way for standardized, miniaturized models well-suited to drug screening, high-content imaging, and integration into multi-organ OoC systems. Bioprinted constructs offer uniformity that is particularly critical in drug and toxicity screening but also in more complex setups where precise tissue ratios (such as between heart and kidney) and microenvironmental conditions influence inter-organ signaling[23,24,36].

Importantly, biofabrication strategies such as ours can support the next generation of *in vitro* disease models. As the field advances, printing technologies are expected to enable the inclusion of endothelial networks, extracellular matrices, and spatially defined co-cultures, further mimicking tissue architecture and physiology[37]. These capabilities are essential for replicating the dynamic, multicellular interactions that underlie human organ development, pathophysiology, and drug response, which are key parameters for both mechanistic studies and personalized medicine.

The power and pitfalls of Organ-on-Chip systems

OoC platforms represent an increasingly important step forward in modeling complex physiology in a controlled environment. Our work demonstrates that combining cardiac and renal organoids in a single microfluidic platform maintains their individual viability and functionality, while potentially enabling the exploration of reciprocal interactions (Chapter 3). Importantly, the evidence of indirect cardiac dysfunction following renal injury described in Chapter 5 suggests that our model captures essential features of CRS type 3 such as early cardiac endothelial impairment driven by systemic inflammation, oxidative stress and exposure to uremic toxins[38–40], opening a door to integration with OoC platforms to perform mechanistic studies and drug testing in a more physiologically relevant context.

However, OoC technologies are still in a developmental phase. Standardization of chip design, scalability for industrial applications, and long-term culture stability remain key limitations. For example, the fabrication of chips often relies on tailored processes and materials, limiting reproducibility and their widespread usage. Furthermore, integrating sensors for real-time readouts, such as impedance for cardiac contraction or markers of glomerular filtration as in the context of this thesis, poses engineering challenges[41–43]. Collaborative efforts, both in the EU (CEL-NEC, NEN) and nationally (partners in hDMT), are working toward standardized chip designs and interface formats[44]. At the forefront of OoC innovation in the Netherlands is also the newly established LUMC OoC center of our department and the NWO-funded LSRI at the University of Twente. Such initiatives are crucial for achieving industrial scalability and interoperability between systems developed by

different labs. These partnerships facilitate the development of integrated systems, promote standardization, and support researchers in advancing human-relevant disease models.

To actuate the full potential of OoC systems, interdisciplinary collaboration is essential. Advances in materials science, sensor technology, and computational modeling can be synergistically applied to develop modular, plug-and-play systems suitable for accurate disease modeling high-throughput screening.

Modeling inter-organ crosstalk *in vitro*: the cardiorenal co-culture system

A significant contribution of this thesis is the development of a static co-culture platform using human hiPSC-derived kOs and cMTs to model the cardiorenal axis described in Chapter 5. This system was designed to capture the interconnected nature of heart-kidney interaction and provide an easier and more accessible alternative to microfluidic-based setups.

The observed reduction in contractility and disruption in endothelial integrity of cMTs rather than myofibrillar integrity upon exposure to injured kOs mirrors the progressive cardiac dysfunction seen in CRS type 3[38]. To our knowledge, this is the first demonstration of renal injury-induced cardiac dysfunction in an entirely human organoid-based platform, offering a unique tool for dissecting reno-cardiac signaling in CRS. Transcriptomic profiling of the co-cultured tissues revealed activation of inflammatory and stress-related pathways, including upregulation of NF-κB-mediated cytokine signaling, oxidative stress responses and extracellular matrix remodeling genes. The model allows the

controlled investigation of acute reno-cardiac signaling and supports the *in vitro* recreation of hallmark pathways outlined in Chapter 2. Our findings offer direct evidence that, independent of hemodynamic coupling, paracrine factors released by injured-kOs are sufficient to precipitate cardiac dysfunction, a core characteristic of CRS. In addition, downregulation of endothelial-associated transcripts points to compromised vascular-like function as an amplifying mechanism of inter-organ signaling. The system recapitulates reno-cardiac signaling in a reductionist, human-relevant model and underscores the importance of integrating multiple organ systems to study systemic diseases. As a standalone or complement to more complex OoC platforms, this model represents a robust tool to investigate mechanistic inter-organ signaling, test interventions and identify biomarkers of disease propagation.

The role of AI and computational modeling in organ system design

As complexity increases in both biological models and the data they generate, the use of advanced computational tools will become indispensable. The integration of artificial intelligence (AI) and machine learning (ML) into organoid research holds potential to transform (disease) model design, predictive toxicology (*e.g.* through the combination of *in silico* models) and data analysis, overcoming manual and time consuming procedures to improve reliability of data and bypassing subjective and operator-dependent results[45–47].

For example, AI algorithms can aid the analysis and quantification of images through automated segmentation as implemented in Chapters 4 and 5. Moreover, they can analyze multi-omics data (genomics, transcriptomics, metabolomics) to identify biomarkers, classify disease subtypes, or predict

organ-specific responses to damage. In the context of CRS, integrating such datasets from co-culture platforms could unveil complex underlying pathways and guide targeted therapy. Additionally, ML models can optimize fluidic parameters in chip design, or even assist in quality control during tissue fabrication. Looking forward, closed-loop systems that combine real-time sensing, AI-based analysis and adaptive control of perfusion or stimulation may open the possibility for a new era of smart OoC platforms[48,49].

As AI integrates into biomedical research reshaping our way of doing science, its use must be pursued ethically. While ML offers the power to accelerate discovery and refine model design, responsible use ensures that human insight, equity, and transparency remain central to scientific progress[50].

Biological and technical considerations for future research

Beyond technological challenges, future advances of these *in vitro* models must address underexplored biological variables. For instance, sex differences in hiPSC-derived lines have often been overlooked, despite rising evidence that cardiac and renal pathophysiology exhibit sex-specific traits. In the context of this thesis, experiments mainly featured female cell lines. Incorporating a balanced panel of male and female hiPSC lines or even XO lines, as well as considering hormonal influences, will be critical for generating inclusive and accurate models and study sex-specific biological differences[51–56]. Ethnicity and ancestry are also variables which receive relatively little attention because of poor annotation of these parameters.

Moreover, in the context of multi-organ systems, tracing the origin of secreted signals in co-culture systems is challenging. Future systems could lever-

age engineered cell lines with *e.g.* barcoded or tagged secretomes, allowing precise attribution of paracrine signals to their source tissue[57]. Combined with high-resolution proteomics, single-cell transcriptomics and functional blocking experiments, such strategies may deconvolute the signaling networks behind CRS progression. This level of resolution would be essential to dissect inter-organ communication.

On the technical side, the static co-culture platform presented in Chapter 5 provides clarity on paracrine signaling but lacks hemodynamic cues such as flow, shear stress and periodic pulsing, all of which modulate both cardiac and renal physiology *in vivo*. Beyond these flow-related limitations, next-generation approaches could help address other current biological and technical hurdles. The incorporation of vascularization and perfusable microenvironments could enhance organoid maturation and nutrient exchange, while standardized differentiation protocols and embedded microsensors would improve reproducibility and enable continuous functional monitoring. Together, these innovations would advance *in vitro* systems toward more physiologically relevant and predictive multi-organ models. Integrating this model into dynamic OoC system such as the one presented in Chapter 3 would be the natural next step in the advancement of this work, allowing for the simultaneous capture of soluble, mechanical and metabolic cardiorenal interactions. Engineering advances like modular chip architectures, or standardized fluid interfaces and embedded sensors for continuous readout, will also be crucial for enabling reproducible, multi-parameter data acquisition.

Finally, scaling up model complexity must be balanced with accessibility. The use of low-cost, open-source bioprinting and fabrication approaches,

as demonstrated in Chapter 4 of this thesis, will be key to ensuring that advanced multi-organ systems are available to a broad range of laboratories. Extending access to these tools may allow the field to accelerate both fundamental discoveries (*e.g.* in the developmental accuracy in which organoids are generated and their physiological relevance) and translational applications, a goal that underpins the broader vision of replacing less predictive animal models with ethically and more human-relevant platforms.

Conclusion

The work presented in this thesis shows how human iPSC-derived organoids and OoC technologies are maturing into powerful platforms capable of modeling complex inter-organ interactions. By combining biological relevance with advanced biofabrication and engineering technologies, these systems hold immense potential for advancing our understanding of multi-organ diseases like CRS (Figure 1).

While technical and biological limitations remain, this field is rapidly evolving and innovations in areas beyond biology are expected to further enhance physiological relevance and predictive value of our models. Ultimately, the shift toward human-relevant, scalable, and ethically sustainable models marks a pivotal transformation in preclinical research, one in which this thesis has played an active part.

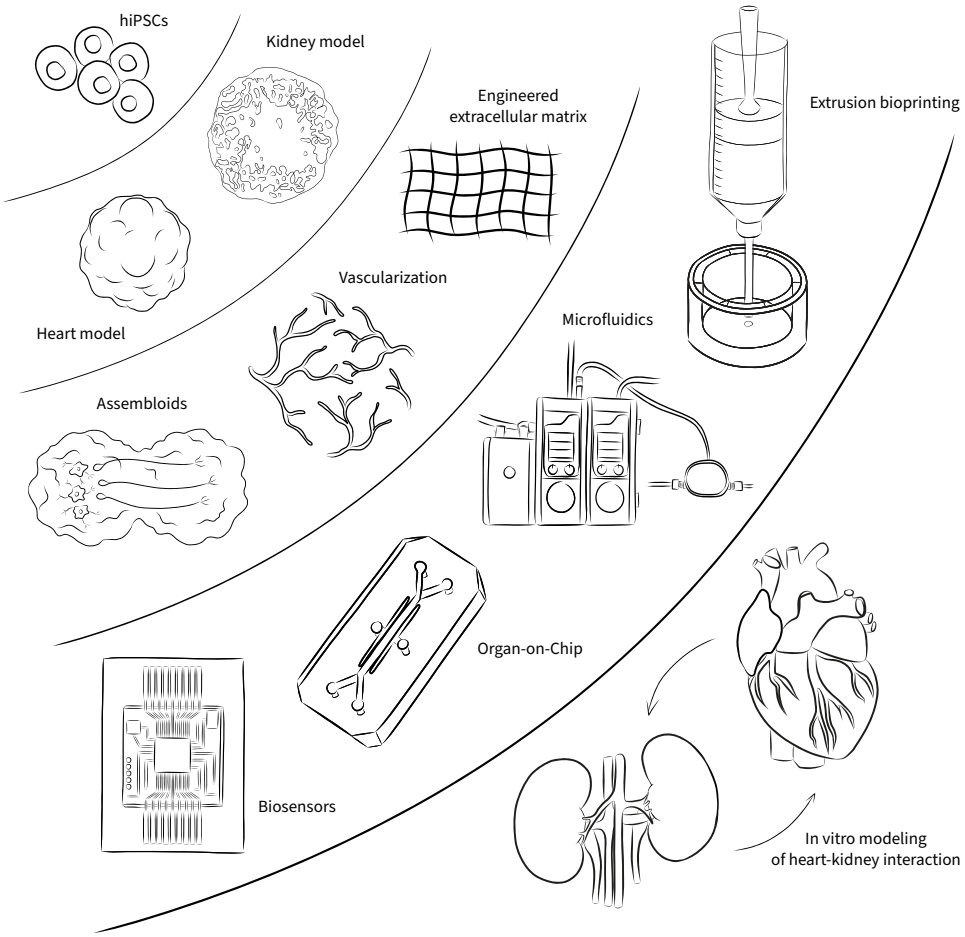


Figure 1. From simplistic hiPSC-derived single-organ models to advanced *in vitro* modeling of heart and kidney interaction.

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