Glomerular filtration barrier on instrumented microfluidic chip

Context:

The increasing prevalence of Chronic Kidney Diseases (CKD) is becoming a worldwide public health issue, as the only treatments of end-stage kidney failure are not only costly but also rely on heavy treatment such as dialysis or kidney transplantation. Since the renal glomerulus is the first structure to perform the blood filtration, it is the primary target in the case of kidney injury.

To better understand the physiology and physiopathology of the glomerulus, there is a need for a new generation of *in vitro* models. Indeed, current *in vitro* models do not reproduce accurately the *in vivo* physiology of the glomerulus and the animal models do not only reproduce poorly human physiology but also suffer of ethical issues. A new category of in vitro systems, known as MicroPhysiological Systems (MPS) or Organs-on-Chip was introduced 10 years ago by Huh *et al.*[1] While MPS represent a very promising technology to enhance the physiological relevance of *in vitro* models thanks to the microfluidic technologies[2], they still lack of two majors *in vivo* physiological features: (i) mature cells expressing the specific markers of interest and (ii) a basement membrane, an extracellular matrix membrane playing an important role in the glomerular filtration[3].
Further advancement of MPS should also allow to get real-time readouts, thanks to integrated sensors, allowing for continuous monitoring of the *in vitro* model and its dynamic effects. To date, existing MPS mimicking the glomerulus, do not implement all three of these aspects or focus on the use of cells that express a better phenotype than glomerular cell lines. The development of a new glomerular filtration barrier-on-chip integrating these different aspects would not only facilitate to perform permeability assays but also help to model and better understand glomerulopathies.
During her PhD work between CoRaKiD, LRS and GeePs, Manon Miran (defence planned in December 2025) studied the assembly of type IV collagen to generate basement membrane and differentiated induced pluripotent stem cells (iPSCs) into glomerular cells. During her stay at the University of Tokyo in the laboratory of Professor Nishikawa in Summer 2024, she differentiated iPSCs into stromal cells to obtain glomerular mesangial cells, which are hypothesized to play an important role in the glomerular physiology. This project aims to deepen this collaboration and develop a complex physiological model of glomerulus.

Objectives and description of the proposal:

The main aim of this PhD thesis proposal is to develop a sensor-integrated microfluidic platform reproducing the glomerular filtration barrier. The microfluidic device that will be optimized during this PhD thesis is based on a prototype that has been previously developed by the current PhD student (Figure 1).

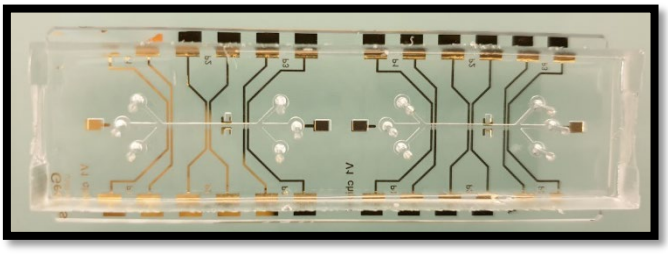

Figure 1: First prototype of the designed microfluidic platform

Building upon this previous research, this proposal introduces a groundbreaking approach with three objectives, addressing critical gaps and pioneering a new direction in the field:

- Objective 1: Generation and characterization of an *in situ* membrane

First, an alginate-based extracellular matrix (ECM) protein will be extruded within the microfluidic device using the methods developed by Onoe *et al*.[4] The key parameters influencing the membrane thickness such as the geometrical features of the channels, the flow rate and the hydrogel viscosity

will be investigated. The composition of the membrane will be also tuned by adding missing ECM proteins or growth factors. The permeability of the membrane will be characterized by impedance spectroscopy and by optical methods using different molecular fluorescent dyes of different weights.

- Objective 2: Generation of a glomerular filtration barrier within the microfluidic chip

Glomerular cells (endothelial cells and epithelial cells called podocytes) will be differentiated from induced pluripotent stem cells (iPSC) using differentiation protocols, which have been optimized at CoRaKiD (Figure 2).[5] Immature glomerular cells will be seeded respectively on each side of the hydrogel membrane and matured under perfusion culture to form a physiologically relevant glomerular filtration barrier. The cell seeding protocol within the microfluidic will be optimized based on the preliminary work currently undertaken. The formation of confluent cell layers on each side of the membrane will be assessed by impedance spectroscopy and by optical methods. The PhD student will carry out these experiments at CoRaKiD in close collaboration with Nishikawa Lab for the glomerular cells, LRS for the impedance spectroscopy methods and with GeePs for the tissue engineering using the microfluidic device.

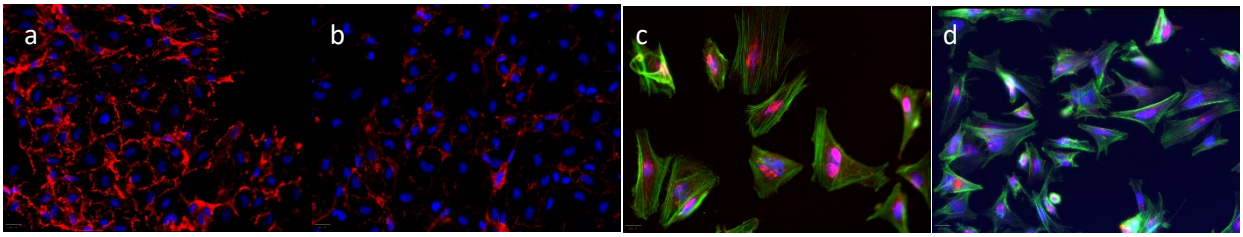

Figure 2: Immunostaining pictures of iPSC-derived endothelial-like cells (a,b) and iPSC-derived podocytes (c,d) on different matrices

- Objective 3: Addition of stromal cells within the culture systems

Based on the expertise of Nishikawa lab at the University of Tokyo in iPSCs differentiation into kidney cels, the PhD student will add a third cell type, glomerular mesiangial cells, within the culture model. The addition of this cell type is expected to change the phenotype of other glomerular cells present within the culture system. The aim, here, is to understand the combined effects of the triculture under perfusion on the glomerular filtration barrier permeability and its microenvironment. The dynamic of these effects will be monitored in real-time with impedance spectroscopy. Additionally, the phenotype of the cells under these different conditions will be assessed.

Candidate:

We are looking for a candidate with a Master degree in bioengineering, cellular biology or biochemistry, who is able to work in an interdisciplinary environment. Experience in microfabrication, cell culture with induced pluripotent stem cells, RT-PCR and immunofluorescence will be appreciated.

Bibliography :

1. Huh, D. *et al.* Reconstituting Organ-Level Lung Functions on a Chip. *Science* **328**, 1662–1668 (2010).
2. Bhatia, S. N. & Ingber, D. E. Microfluidic organs-on-chips. *Nature Biotechnology* **32**, 760–772 (2014).
3. Miner, J. H. Glomerular basement membrane composition and the filtration barrier. *Pediatric Nephrology* **26**, 1413–1417 (2011).
4. Onoe, H. *et al.* Metre-long cell-laden microfibres exhibit tissue morphologies and functions. *Nature Mater* **12**, 584–590 (2013).
5. Musah, S. *et al.* Mature induced-pluripotent-stem-cell-derived human podocytes reconstitute kidney glomerular-capillary-wall function on a chip. *Nat Biomed Eng* **1**, 0069 (2017).