MIcrofluidic glomerular filtration barrIer-on-Chip with Integrated Sensors (ICIS)

Context:

The prevalence increase of Chronic Kidney Diseases (CKD) is becoming a worldwide public health issue because 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 main target in the case of kidney injury. In order to 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. New *in vitro* systems, called MicroPhysiological Systems (MPS) or Organs-on-Chip have been introduced, 10 years ago, by Huh *et al.*[1]. If 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) basement membrane, an extracellular matrix membrane playing an important role in the glomerular filtration[3]. Further enhancement of MPS should also allow to get real-time readouts, thanks to integrated sensors, in order to monitor the *in vitro* model and the dynamic effects. Up to now, MPS mimicking the glomerulus, do not implement all these three aspects[4,5] or focus on the use of cells that express a better phenotype than glomerular cell lines[6,7]. The development of a new glomerular filtration barrier-on-chip integrating these different aspects will not only help to perform permeability assays but also to model and understand glomerulopathies. This project is based on an existing collaboration between the laboratories CoRaKiD, LRS and GeePs.

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 based on a prototype that has been developed by the former intern student between LRS and GeePs (Figure 1).

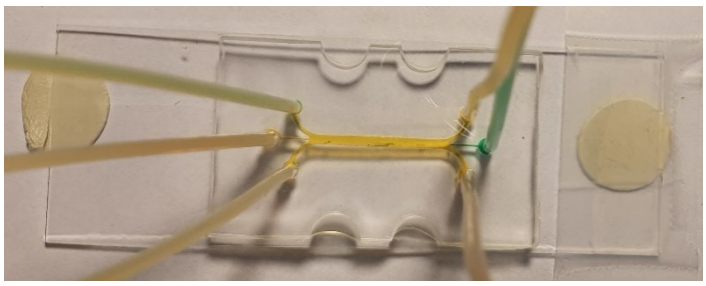

Figure 1: First prototype of the designed microfluidic platform

Preliminary work on the electrode integration is currently on going. Based on this preliminary work, this proposal is composed of three objectives:

- Objective 1: Engineering a physiological relevant glomerular basement membrane within microfluidic device

Currently engineered basement membranes (BM) do not properly mimic the biophysical and biochemical properties[8]. The objective, here, is to develop a hydrogel containing type IV collagen and laminin which are major components of the glomerular BM and type I collagen. The hydrogel formulation is currently under investigation (CoRaKid and GeePs). The PhD student will characterise

the properties of the hydrogel self-assembly on functionalized surfaces, using atomic force microscopy. The hydrogel will be then extruded within the microfluidic device using the methods developed by Onoe *et al*.[9] in order to form a membrane.

- Objective 2: Generation of physiological glomerular cells from stem cells within microfluidic device

As glomerular cell lines (endothelial cells and epithelial cells called podocytes) present phenotype limitations especially after long period of culture. In order to obtain a more physiologically relevant cell phenotype, induced pluripotent stem cells (iPSC) have shown promising results. The PhD student will differentiate iPSC into immature glomerular cells according to published differentiation protocols[10,11], that are currently optimised at CoraKid (Figure 2). Immature endothelial and epithelial cells will be seeded respectively on each side of the hydrogel membrane. They will be then matured under perfusion culture in order to form a physiologically relevant glomerular filtration barrier. Its permeability property will be assessed thanks to: (i) optical methods with fluorescent labelled-proteins and (ii) impedance spectroscopy methods with the electrodes[12] (CoRaKiD and LRS). PhD student will carry out these experiments in CoRaKiD in close collaboration with LRS for the impedance spectroscopy methods and with GeePs for the tissue engineering with microfluidic device.

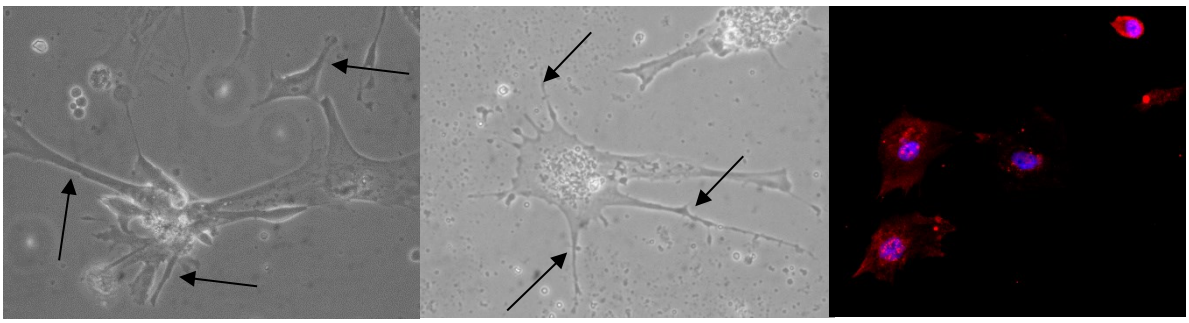

Figure 2: Left and center: Podocyte-like cells differentiated from iPSCs exhibiting possible foot process (black arrows), a podocyte feature and right: immunofluorescence of Podocyte-like cells staining synaptopodin (cytoskeleton associated protein) in red and nuclear DNA in blue

- Objective 3: Reproduction of a glomerulopathy with the glomerular filtration barrier-on-chip

Based on the established expertise of CoRaKid in the pathophysiology of glomerular diseases[13–16], particularly, membranous nephropathy where increase permeability of the glomerular filtration barrier is induced by antibodies to podocytes (the external cell layer of the barrier), the PhD student will leverage this platform (Figure 3) to model the glomerular events of the disease using the serum (or purified Immunoglobulins) from the patients with membranous nephropathy of from healthy controls or patients with a different mechanism of disease (IgA nephropathy).

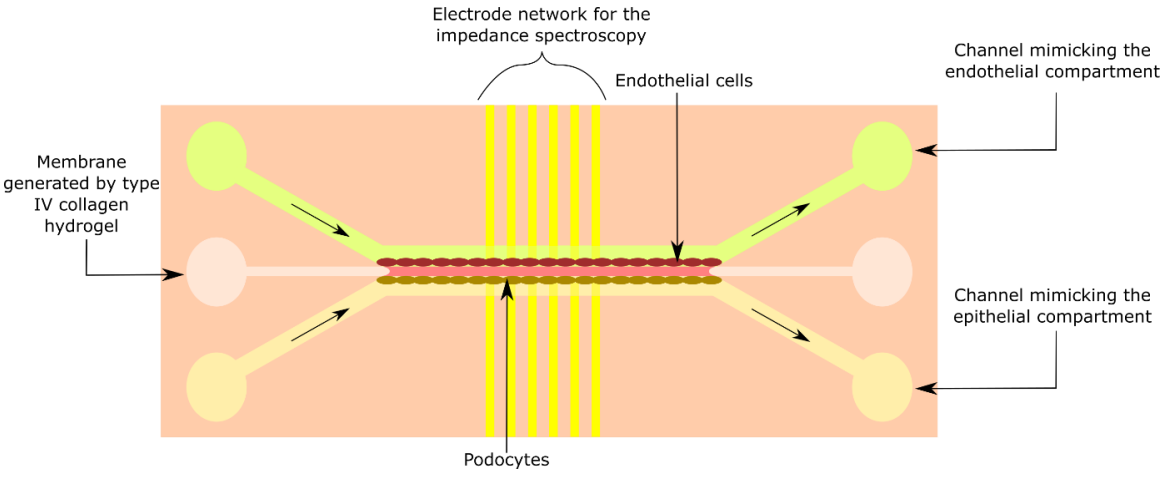

Figure 3: Top-view of the microfluidic device reproducing the glomerular filtration barrier

The aim, here, is to understand the combined effects of the permeability factors (antibodies) and the glomerular microenvironment. The dynamic of these effects will be monitored in real-time with impedance spectroscopy.

Candidate:

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

Bibliography : The publications from the supervisors are underlined.

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