Perfused glomerular microvascular unit. A glomerular pathophysiology model

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Introduction

The study of the pathophysiology of kidney diseases is pivotal for understanding mechanisms involved in glomerulopathies and their evolutions. Nowadays, these studies are based on murine or cellular models with some limitations linked to species characteristics, involvement of cell interaction or cross-talk and/or involvement of fluid shear stress.

Using a tissue engineering approach, the project will reproduce the glomerular architecture by performing a glomerular perfused microfiber.

Materials & Methods

Human immortalized glomerular cell lines: podocytes and endothelial cells (EC) were used. Fabrication of a cellularized microfibre was based on our biofabrication method [1]: an alginate shell was made by soaking alternatively a microcapillary in alginate sodium 2% and calcium chloride 200mM. Then, the cellular solution compound with 35.10^6/mL GEC and type I rat collagen (concentration = 5mg/mL) was injected inside the microcapillary. After 18 hours at 33° C, the alginate shell was removed linked to compaction phenomenon. The microfibre was incubated at 37° C.

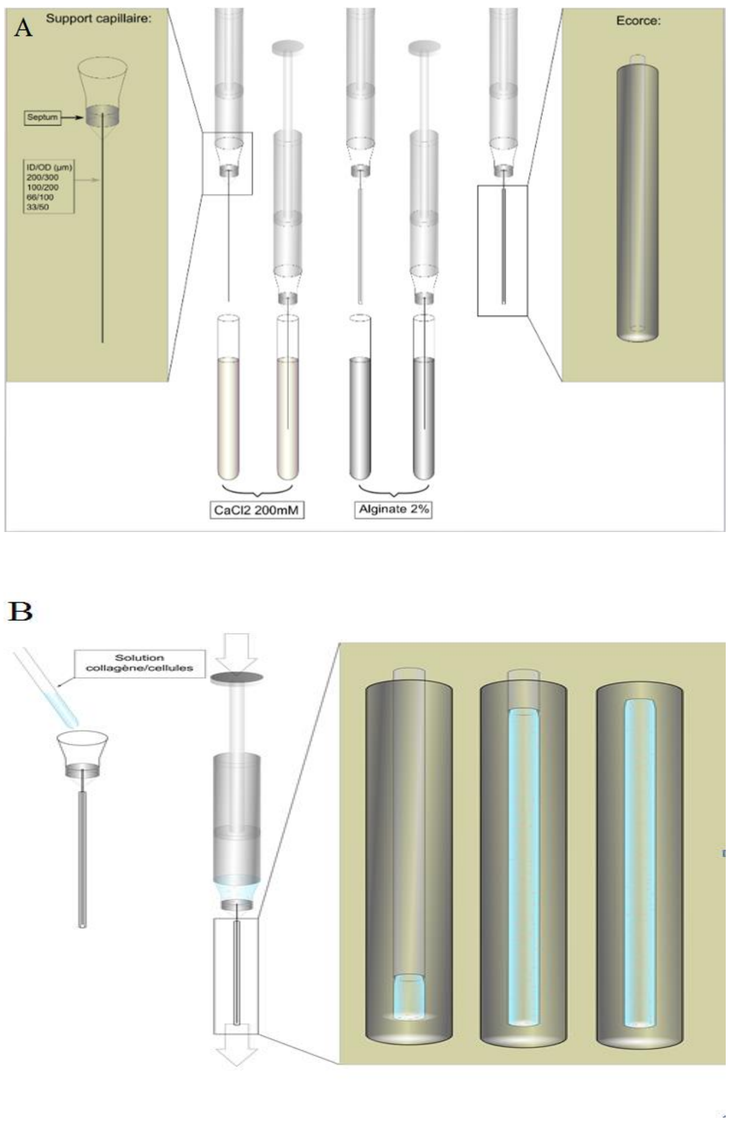


Figure 1: Microfabrication method [1]

After five days at 37° C, the microfibre was seeded with 5.10^4 podocytes/mL to create an external layer of podocytes. The microfibre was incubated at 37° C for 14 days to achieve the differentiation of podocytes.
Cells and 3-dimensional (3D) matrix have been characterized by immunofluorescence (IF) with confocal analysis and Western blot (WB). Optical microscopy was performed to study microfiber compaction and contraction. We also analyzed cell viability and cell metabolism within the 3D construct.

Results

Using the microfiber method developed in the unit, we repeatedly obtained a cellularized microfiber sorting human glomerular cells in 3D. Around a central structure made of collagen I, we successively found the internal layer composed by EC, the neoformed glomerular basement membrane rich in collagen IV and the external layer of podocytes.

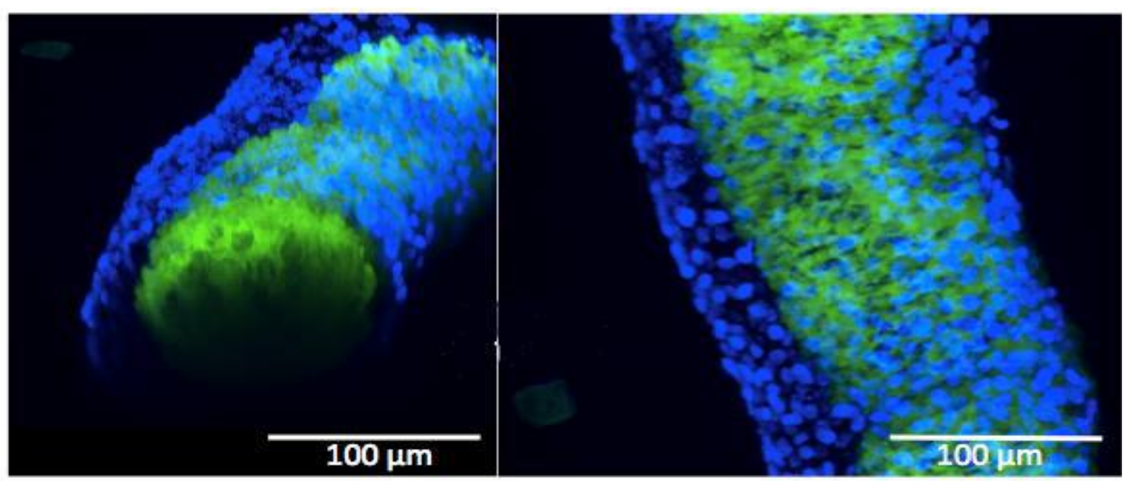


Figure 2: 3D spatial conformation of cellularised microfibers. collagen (green) and nuclei (blue) were stained and analysed by confocal microscopy. GEC migrated to the periphery of the microfibres and were based on a collagen I matrix

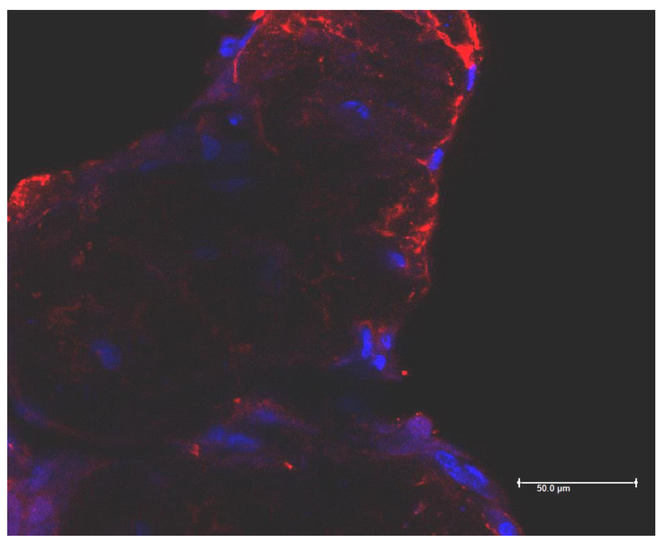


Figure 3: Immunofluorescence staining of Collagen IV. A microfibre seeded with 50 000 cells/mL was stained to reveal the expression of collagen IV. Nuclei (blue) and collagen IV (red) were mostly located at the periphery of the microfibre. Collagen IV was clearly expressed around cells.

Cell concentration, optimal seeding time and role of physical stresses were appreciated and modulated to obtain the microfiber.

Cell viability and cell phenotype were confirmed by IF and WB analysis: expression of specific proteins, vWF, PECAM and VEGFR2 for EC and nephrin, synaptopodin and podocin for podocytes.

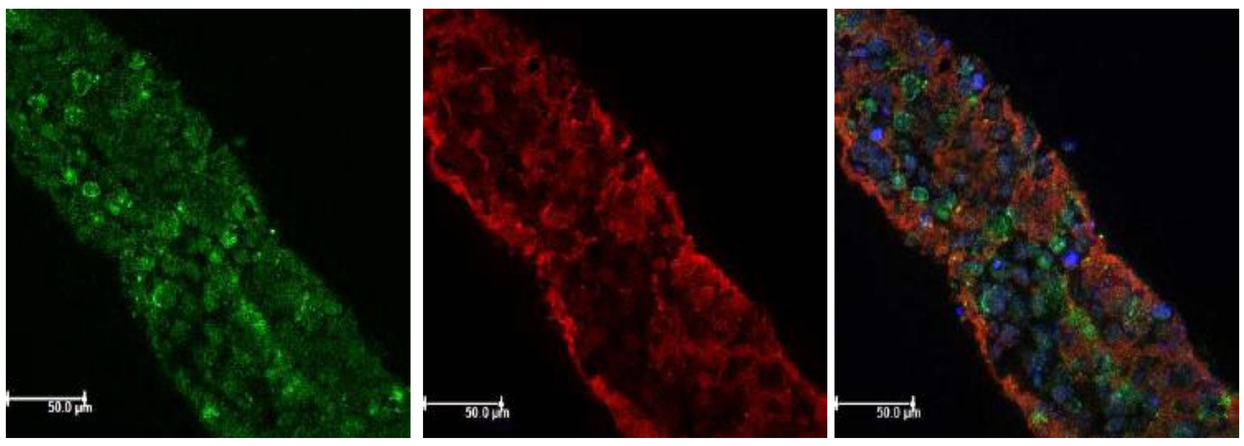
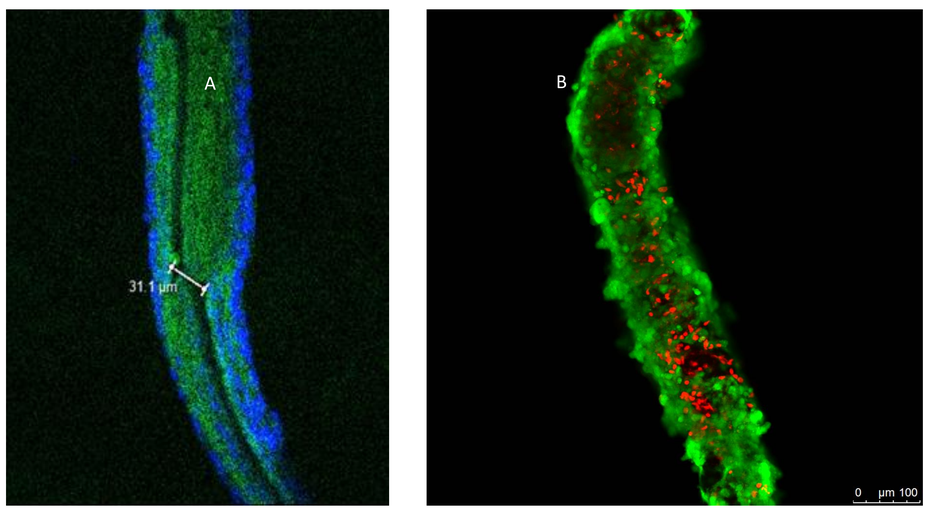


Figure 4: Immunofluorescence double staining of PECAM-1 and Podocin. Nuclei (blue), podocin (red), PECAM-1 (green) were expressed. Podocin expression was mostly situated on the edge of the microfiber while PECAM-1 was locally expressed at the internal layer.

Conclusion

In summary, with this microfiber technique, endothelial cells and podocytes were better differentiated than in culture dishes, and produced a differentiated GBM. Glomerular fluid stresses and glomerular pathophysiological conditions will be shortly simulated in the glomerular microfibers using the microperfusion method. Nowadays, a central lumen formation by femtosecond laser is obtained with cell characteristic maintenance.



A glomerular microvascular network will allow us to study cell interactions in a 3D system and increase our knowledge on the glomerular pathophysiology.

Funding: Agence de Biomédecine

[1] An easy-to-use and versatile method for building cell-laden microfibres. J. Kalisky, J. Raso, C. Rigothier, M. Remy, R. Siadous, R. Bareille, J.C. Fricain, J. Amedee-Vilamitjana, H. Oliveira, R. Devillard. Sci Rep 6 (2016) 33328.