Dynamics of endothelial engagement in complex 3D microstructures built by two-photon photopolymerization

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Context : The fundamental understanding of endothelium-extracellular matrix interactions during the formation of new blood vessels meets considerable medical needs. The 3D geometrical organization of matrix fibers is complex, and modified in many pathophysiological conditions, like in tumor microenvironments which favor the formation of tortuous and disorganized vessels. Our group focuses on early steps of angiogenesis, where "leader" tip cells invade extracellular matrix upon growth factors gradients, leading trailing endothelial cells (stalk cells). Tip/stalk transient specification is governed by Dll4/Notch signalling, with tip cells emitting exploratory filopodia important for angiogenesis, while stalk cells are devoid of filopodia.

We have previously identified 3D microstructures triggering the formation of endothelial filopodia evocative of a tip cell phenotype (Figure 1). Filopodia were formed with or without Vascular Endothelial Growth Factor, suggesting that geometry alone might lead to tip cell phenotype. Our 3D microstructures are realized by two-photon photopolymerization, which allows to build structures at a subcellular scale thanks to the confinement of the excitation to the focal volume (μm^3). We are now building more complex 3D structures in order to study early steps of endothelial multicellular engagement, and filopodia dynamics coupled to collective migration. Our system based on two-photon photopolymerization provides an ideal tool for modifying at will the 3D geometries and stiffness characteristics of the tunnels, and to understand finely the dynamic adaptation of vessel formation to the microenvironment.

Objectives :

This internship aims to optimize the creation of tunnel-like 3D microstructures promoting multicellular engagement and collective migration guided by patterns with varied properties (like stiffness, spatial organization or protein coating). The cell migration mode as well as the formation of filopodia in response to the local geometrical and mechanical constraints will be characterized from a biology perspective (stainings, pharmacology) and from a physics perspective (migration speed, dynamics of filopodia formation, exerted forces).

The intern should have basic knowledge of cell culture techniques. During the period of the internship, he/she will perform cell culture (HUVECs), live imaging (spinning disk, two-photon, lattice light sheet microscopes), stainings (nuclei, cytoskeleton, E-Cadherin, focal adhesion proteins, ion channels, actin regulators…) (collaboration C. Monnot, Collège de France). The intern should demonstrate some interest in microfabrication as part of the work will be dedicated to the fabrication and functionalization of microstructures by two-photon photopolymerization.

Depending on the intern's profile and interests, the project may also include an aspect of :

- Microfabrication : participation in the design of new architectures (use of CAD software like Sketchup, optimization of slicing and laser parameters for fabrication) and characterization of the resulting structures (SEM and AFM).
- Chemical functionalization : optimization of the spatial patterning of chemical coating in the structures. Coatings include PEG (anti-adhesive), extracellular matrix proteins (collagen, fibronectin, laminin…), angiogenic signalling pathways molecules (VEGF, Dll4…).

- Image analysis : use of Python/Keras for automated segmentation of cellular components via CNN (Convolutional Neural Networks), participation in the annotation of 3D images, in the optimization of the architecture and parameters of the neural network, in the evaluation of performances and comparison with other existing tools.
- Modeling : use of Comsol Multiphysics to model the forces exerted by the cells in the microstructures based on the local deformations.
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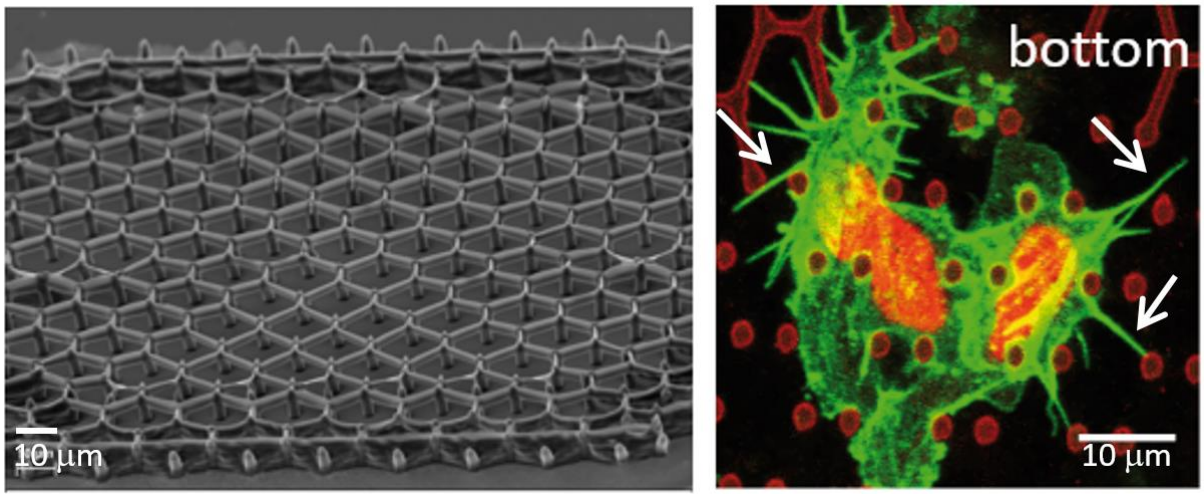

Figure 1 : Arrays of hexagons on pillars built by two-photon microscopy (left), leading to the generation of endothelial filopodia in the bottom plane (arrows) (HUVECs cells, actin/nuclei labeling, right).