Practicals

N°68: Cellular assembly on rigidity-patterned substrates

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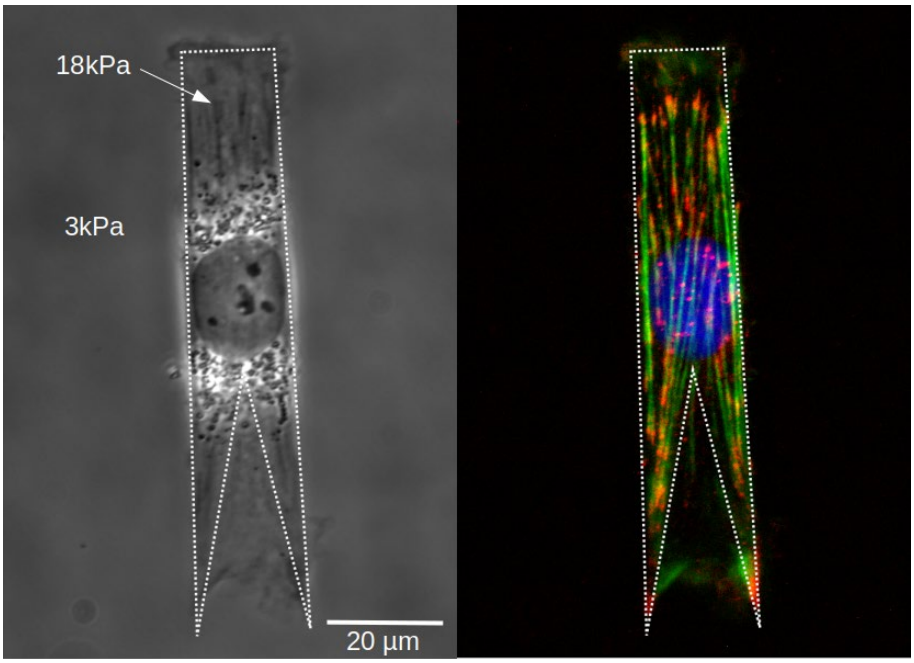
The study of mechanical interactions between cells and their substrates have become an area of intense study and seen as a promising pathway toward tissue engineering and drug discovery[1]. Cells are submitted to different types of external mechanical stimuli such as forces exerted by adjacent cells or blood flow. These external forces trigger mechanical stresses that are transmitted through the plasma membrane to the cytoplasm. Therefore, tissue cells could respond to the stresses by several mechanisms including assembly or disassembly of adhesion patches, actin remodeling, and ion channel activation[2]. Cell responses to rigidity appear as essential regulators of various biological functions. Evidence supports that force sensing is crucial during the different steps of cellular life[2] and impacts cell adhesion, contractility, and the subsequent biological processes such as cell migration, differentiation and apoptosis. An alternative to address the impact of rigidity modulations on adhering cells in dependence on the surface density of the extracellular matrix (ECM) is to design soft culture supports (hydrogels) with biomimetic, micron-scaled patterns of rigidity and tunable surface chemistry.

In this practical we will explore the technological processes necessary to create these patterned soft hydrogels. In addition, we will design a hydrogel-based synthetic extracellular matrix with patterns of rigidity using gray leveled lithography. Finally, we propose to seed A549 cell line[3] – a model that represents adenocarcinoma human alveolar basal epithelial cells, extensively studied and used as a tool for lung cancer research – and compare their cellular behavior when exposed to a uniform or patterned rigidity hydrogels coated with a uniform surface density of proteins of the extracellular matrix, or when the proteins of the extracellular matrix are mainly condensed on the stiffer regions of the patterns.



Example of Fibroblast cell grown on a hydrogel patterned with a dunce cap pattern of rigidity. The cell takes the shape of the stiff pattern. Red: vinculin; green: actin; blue: nucleus.

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[1] Kandow C.E., Georges P.C., Janmey P.A. & Beningo K.A. (2007). Polyacrylamide hydrogels for cell mechanics: steps toward optimization and alternative uses. Methods Cell Biol. 83, 29–46.
[2] Ladoux, B., & Nicolas, A. (2012). Physically based principles of cell adhesion mechanosensitivity in tissues. Reports on Progress in Physics, 75(11), 25.
[3] Cooper, J. (2012). A Cell line profile A549. European collection of authenticated cell cultures.