Cellular topology sensing in the extracellular matrix

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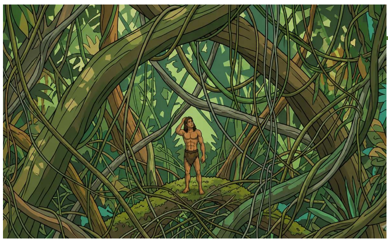
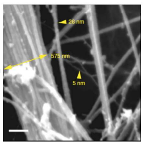
FIND YOUR WAY INTO THE MATRIX

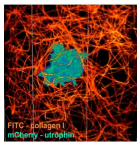


How is Tarzan able to find his way through the meshwork of jungle vines ?

As Tarzan, cells need to navigate through a dense network of fibers of different sizes and different physical and adhesive properties: the extracellular matrix (ECM). Cell migration is a fundamental process involved in tissue homeostasis, immune response but also in devastating diseases such as cancer through metastasis.



Mouse gut basal membrane



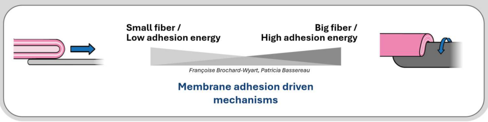
Neutrophil-like human HL-60 cell in a collagen matrix

Physics suggests the existence of two modes of interaction between a membrane and an adhesive fiber

Cells have different receptors on their membrane to sense the ECM proteins surrounding them and they can detect obstacles and topological changes. [1][2] Moreover, previous studies on bacterial infections also reveal two distinct membrane adhesion driven protrusions which are balanced by the fiber diameter and adhesive properties [3][4].

The goal of this project is to understand **how cells perceive and respond to the diameter and adhesive properties of different ECM fibers**

Physical model

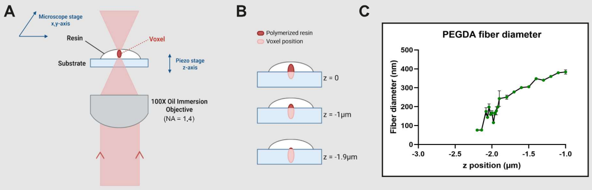


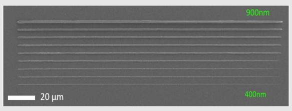
PHOTOPOLYMERIZATION OF NANOMETRIC FIBERS

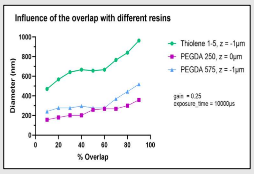
In *vitro* fibers are fabricated using Two-Photon Polymerization (TPP) and a photoactivable resin (Poly(ethylene glycol) diacrylate (PEGDA) or Thiolene) [5].

Precise control of the size and the architecture of the fibers. Micrometric to nanometric diameters are obtained playing on the z position of the voxel, the overlap, the gain and the exposure time of the laser.

Obtention of 75nm to 1µm diameter PEGDA and thiolene fibers using TPP

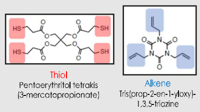


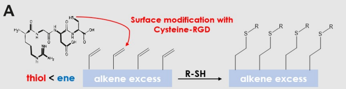




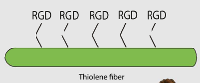
FIBERS FUNCTIONALIZATION WITH ECM PROTEINS

Free alkene functions on the thiolene fiber surface react with a RGD-cysteine peptide through an alkene-thiol reaction





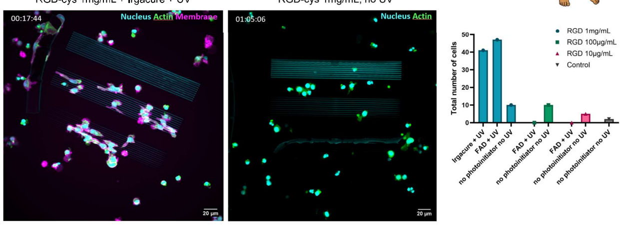
B Representation of the final structure



NEUTROPHIL ADHESION

RGD-cys 1mg/mL + Irgacure + UV

RGD-cys 1mg/mL, no UV



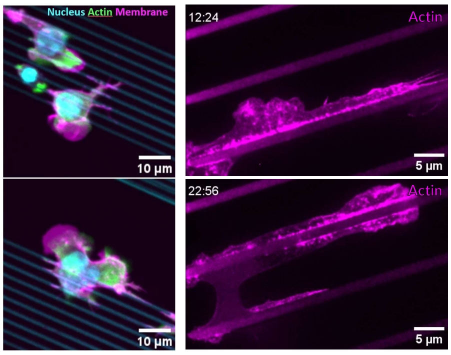
HL-60 derived neutrophil cells **adhere and deform along the RGD-functionalized thiolene fibers.**

Minimal RGD-cys concentration of 1mg/mL for the functionalization to be effective.

INFLUENCE OF FIBER SIZE AND SPACING

3µm-spaced fiber 600nm-diameter

10µm-spaced fiber 2µm-diameter

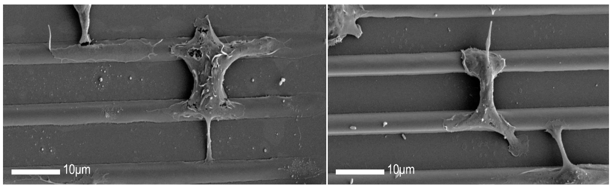


HL-60 derived neutrophils **spread on multiple 3µm-spaced fibers.** For 10µm-spaced fibers they jump from one fiber to another.

Neutrophils seem **more elongated and guided by 2µm-diameter fibers** than by the 600nm-diameter ones.

HIGH-RESOLUTION MORPHOLOGY

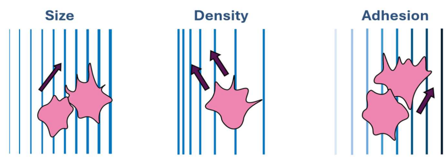
- **SEM: Scanning Electron Microscopy (UBI platform)**



SEM allows to observe cells and their protrusion morphologies with more accuracy. Preliminary tests revealed neutrophils affinity for RGD-functionalized thiolene fibers, spreading all over them. **Protrusions are formed over 10µm-spaced fibers** to fill the gap between two adhesive pattern.

PERSPECTIVES

- Gradient of sizes, spacing and adhesion
- Quantification of cell direction, speed and persistence



References

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