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Titre du stage : Smart interrogation of mechanosensing in T cell activation

Scientific context

Cell/cell interactions are a paradigm in cell biology since they establish, through biochemical and mechanical contacts, the structures of the living, allowing the cell's function to take place and potentially synchronise their action. While the biochemical side has been intensively studied, the mechanics , and its articulation with biochemistry, together termed as mechanotransduction, is a current topic of enormous interest. Its importance is more and more recognized to be instrumental to finely regulate cellular functions. The interface formed between a T cell and an antigen presenting cell during immune response, often called the immune synapse, is a prototypical interaction where mechanotransduction is a key regulating mechanism. . This particular moment is crucial for adaptive immunity as this is when the pathological antigen is recognized by the T cell and the decision is taken whether to mount a response or not. The formation of the immune synapse ultimately leads to the production of inflammatory signals and mutual exchange of signals between the cells, as a prelude to the elimination of the pathogenic condition

Long term objectives of this project

The over-arching goal is to bridge our understanding of molecular mechanics and cell scale mechanical behaviour in the adhesion/spreading dynamics of activation of T cells. Specifically, we shall:

1. Design innovative substrates to manipulate the adhesion : soft, micro or nano patterned elastomers or gels on which cells will spread

2. Measure cell generated forces during and after spreading, on the designed substrates, *via* new modalities of traction force microscopy (TFM), coupled with atomic force microscopy (AFM) and optical tweezers (OT) to record modulation of cell mechanics.

3. Quantify activation levels on designed substrates and under force.

In the frame of this project, on which a PhD student is working in LAI, we propose to help designing and using nano structured, deformable and transparent substrates to perform traction force microscopy, which are compatible with advanced optical imaging and amenable to combination with force measurements using atomic force microscopy and optical tweezers. The student will evaluate the dynamics of spreading of T cells on specific molecules, and if possible, the forces during this process.

We recently put in place protocols for facile production of patterns consisting of an array of functionalized nano-dots with variable pitch and size (range few micrometers to tens of nanometers). These nano-patterns mimic the APC membrane organization and tune cell adhesion and resulting activation. Crucially for the present project, they can be transferred onto soft transparent elastomers to introduce variations in substrate mechanics to be one step closer to a real APC situation. Uniquely, these nano-patterned soft substrates are perfectly compatible with advanced microscopy including TIRF, unlike the other currently available alternatives. These patterned substrates are also inherently suited to perform traction force microscopy (TFM) by following the position of the protein (fluorescent) nano-dots. As such, and containing no nano-bead with may interfere, for example, with optical tweezers set-ups, they are expected to be uniquely compatible with useing TFM and OT simultaneously on the same cell. We also recently set in place the use of calibrated soft polyacrilamide gels, incorporating fluorescent beads for traditionnal traction force microscopy.

→ Expected main outcomes: Spreading and traction data on smart substrates.

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