Studying cell mechanics during early stages of infection using Atomic Force Microscopy and correlative microscopy techniques

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1. INTRODUCTION

The interaction between host cell and pathogens involve ligand-receptor type of binding that favors the signal transduction in order to organize the cell response. This response allows for instance to reorganize the cytoskeleton required for the internalization of invasive microorganisms. On the other hand, the cell can also respond to mechanic stimuli that may control the cell fate as shown by Discher and colleagues who demonstrated that the mechanical properties of the substratum will condition how stem cell differentiate [1]. During the host-pathogen adhesin/receptor interactions as well as mechanical forces are involved and our working hypothesis is that they participate to the regulation of the cell signaling response. To assess this phenomenon, we developed an original approach using correlative microscopy between several types of methods: atomic force microscopy (to measure forces), fluorescence microscopy (including functional dynamics and super-resolution studies), and electron microscopy (for ultrastructural purposes). We will discuss this approach during the presentation.

2. RESULTATS

2.1 Atomic force microscopy - Stiffness 3D mapping

To investigate the mechanical features of the sample, we are using force measurements using the contact mode. In this mode the tip of the cantilever interacts with the sample and the deflection of the cantilever is followed on a photodiode detector. From the extension/retraction curves, one can extract several parameters as the stiffness (young modulus), the adhesion and interaction forces and viscosity. In particular, we are interested in the stiffness. We can segment the indentation curve. For each stiffness map at a given indentation, we can measure the relative stiffness given by averaging the young modulus of one pixel by the sum of the young modulii obtained for the two adjacent pixels on the scan line. This allows to analyze the relative stiffness on 3D maps.

We can for instance analyze the stiffness of the actin structure in a comet tail formed when the bacterium *Shigella flexneri* has gained access to the cytoplasm after its internalization in HeLa cell used as a host. This allows the bacterium to benefit from a propulsive force induced by the pathogen itself. 30 minutes post infection, sample is fixed.

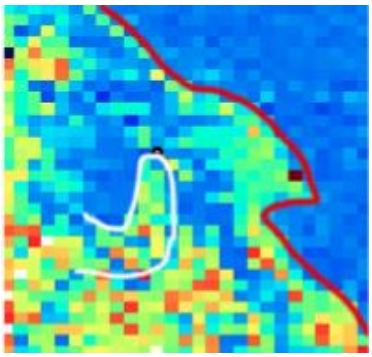

Figure 1. Stiffness mapping ; the pseudo-color codes from soft (blue) to stiff (red). The red line depicts the edge of the cell, the white one the actin tail comet.

2.2 Fluorescence microscopy acquisition

Our AFM setup couple the AFM head to an inverted microscope equipped for TIRF, PALM/STORM acquisition. Hence, it is possible to record including using super-resolution the distribution of molecules tagged

with fluorescence (directly in the case of protein chimera fused to fluorescent protein or stained with antibodies coupled to fluorescent probes). For instance, using photoactivated localization microscopy (PALM) and at a localization precision of 30nm, we can appreciate the distribution of actin on the tail comet of intracytoplasmic bacteria.

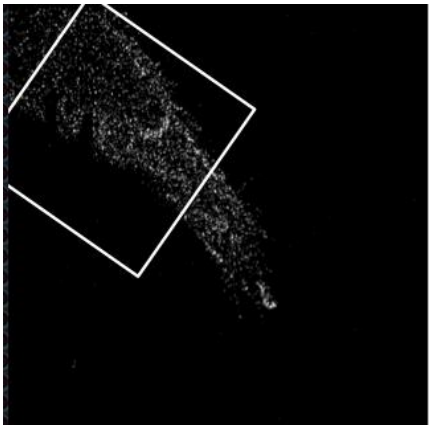

Figure 2. actin-tdEOS distribution in the infected HeLa cell

2.3 Electron microscopy

After force measurement and fluorescence recording, the sample is processed for transmission electron microscopy. 100nm beads are used as fiducial markers for the correlation.

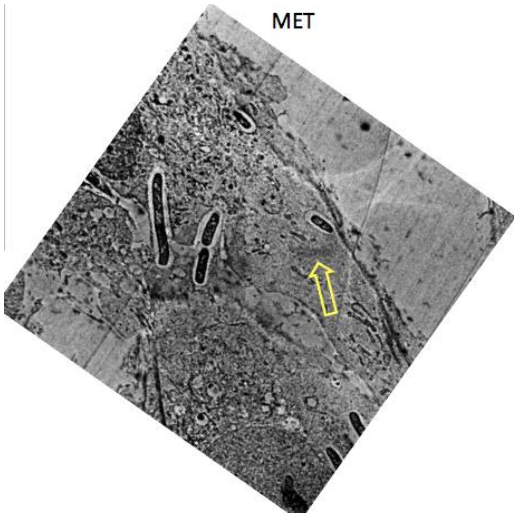

Figure 3. TEM analysis

3. CONCLUSION

We have introduced a correlative approach using atomic force, electronic and light microscopies (CLAFEM). The sample shown herein has been fixed but we will discuss the case of living samples. We will also discuss other super-resolution techniques as RESOLFT/STED or more conventional light microscopy.

REFERENCES

[1] Matrix elasticity directs stem cell lineage specification. Engler AJ, Sen S, Sweeney HL, Discher DE. Cell. 2006 Aug 25;126(4):677-89.