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Extracellular vesicles deformation on surface: Some tracks to limit it

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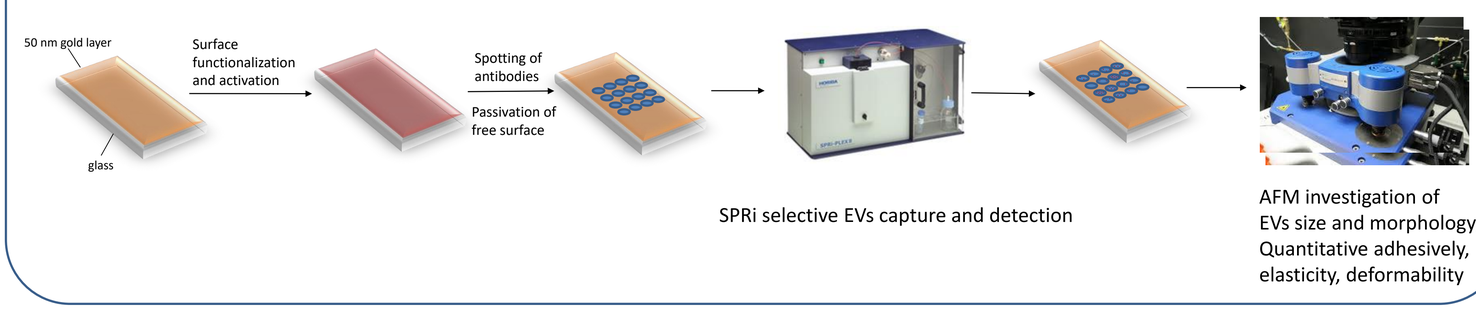
Context:

Despite the booming development of multiple characterization techniques of extracellular vesicles (**EVs**), reliable nanocharacterization of EVs still remains a challenge due to the large variety of their size and cell origin. In this context, our efforts are aimed to the development of a **NanoBioAnalytical (NBA) platform**, which combines several characterization techniques, including **Atomic Force Microscopy (AFM)** – a source of information about EVs metrology. Our NBA platform consists in a biochip, which is biofunctionalized in a multiplexed format through the grafting of different relevant and specific ligands. This biochip behaves like a "EVs smart carrier", since it first enables the biodetection and capture of EVs subsets due to a **Surface Plasmon Resonance** technic, while EVs size and morphology are achieved on the same biochip by AFM afterwards. Nevertheless, EVs are known to be soft and deformable, thus their dimensions and morphology obtained by AFM measurements may vary, among other things, according to support constraints. We intend to create a confident EVs nanocharacterization instrument, thus we optimize both biointerface and AFM mode and parameters in order to limit the deformation of the vesicles and get reliable metrology information.
In current work we exploited thrombin-activated platelet-derived microparticles as model EVs.

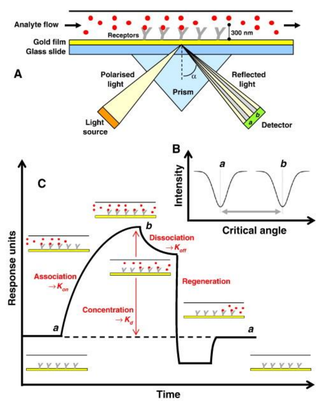
Biochip preparation and NanoBioAnalytical platform principal elements



SPRi selective EVs capture and detection

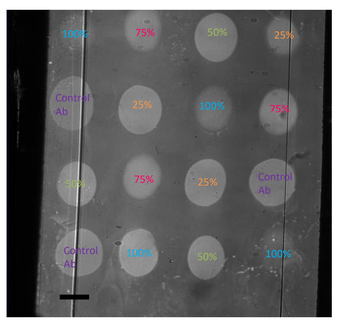
AFM investigation of EVs size and morphology; Quantitative adhesively, elasticity, deformability

Capture and biodetection of EVs using Surface Plasmon Resonance



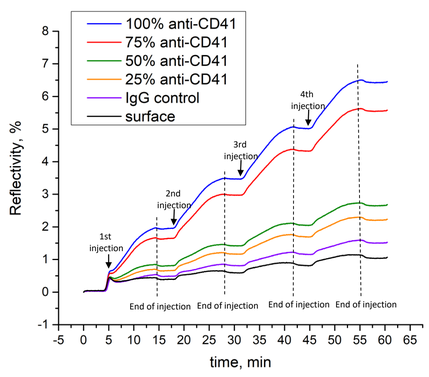
***SPR principle**. A. Setup of a microfluidic SPR biosensor. The configuration includes a light source, a prism and a detector, all coupled to a metal-covered sensor biochip. B. SPR detection involves the difference in reflectivity and the shift of the position of surface plasmon resonance angle. C. Typical SPR response curve (sensorgram) depicting different events on the surface of a biochip.*

➢ Specific antibodies (anti-CD41) were mixed with non-relevant antibodies (A9H12, anti-LAG3) at different proportions before grafting on the biochip

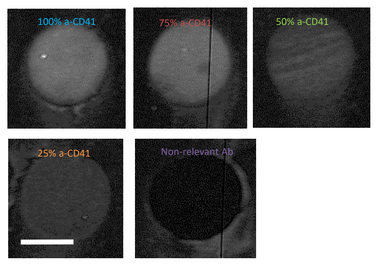


SPRi image of antibodies array on the surface of a biochip before the injection of EVs. Mixtures with different content of anti-CD41 (indicated in % on the image) were spotted on the surface of a biochip. Scale bar is 500 µm.

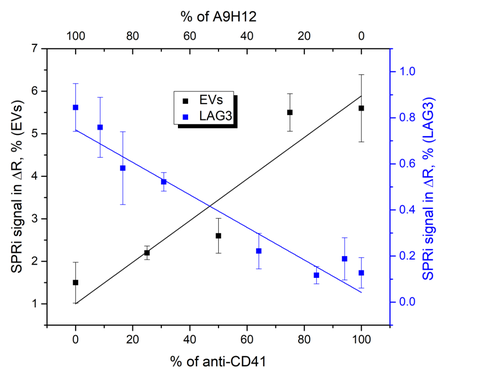
➢ Capture of EVs is proportional to the amount of specific antibodies (anti-CD41) applied during the grafting



SPRi sensorgram of 4 consecutive injections of EVs



SPRi differential images the spots after the injections of EVs. Scale bar is 500 µm

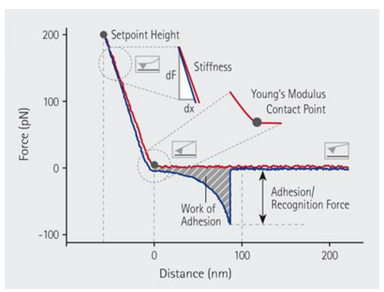


Capture level (represented in reflectivity variation in %) of EVs and LAG3 on the spots with different ratio between anti-CD41 and A9H12 before grafting on the surface

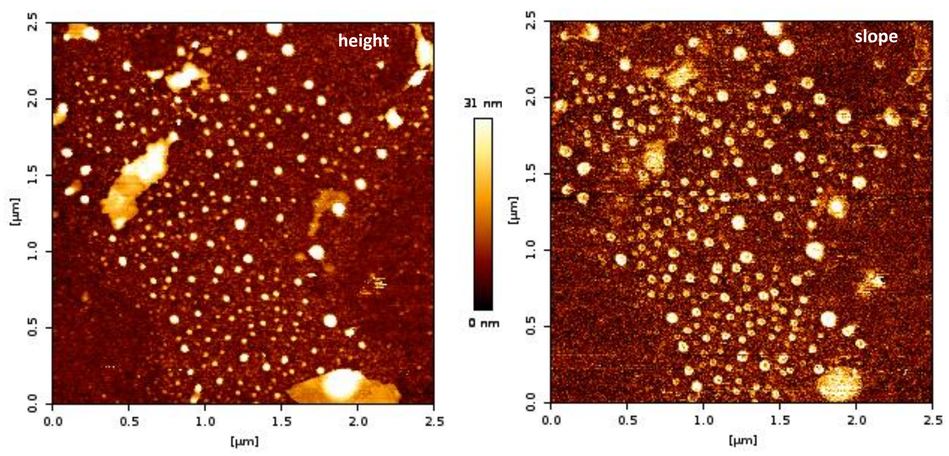
✓ Good correlation between specific antibodies content in solution and the reactivity of biointerface

AFM imaging in QI mode

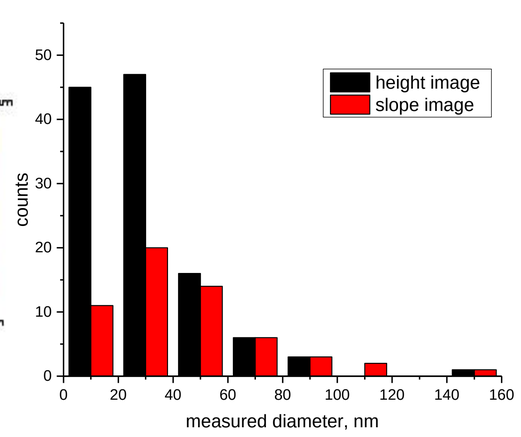
➢ In QI (Quantitative Imaging) mode AFM tip approaches and retracts from the surface at every point. A complete force-distance curve is recorded at every pixel, allowing extraction of a comprehensive set of sample properties. Zero lateral forces and low peak force prevent the sample from damaging and deformation



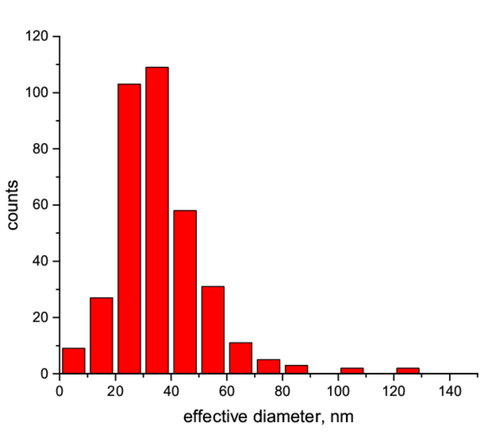
➢ Simultaneous analysis of both height and slope images allows taking into account the smallest particles without confusion with the background noise coming from the roughness of gold surface.



Left: height and right: slope (stiffness) AFM images of the same zone of EVs captured on a spot of anti-CD41



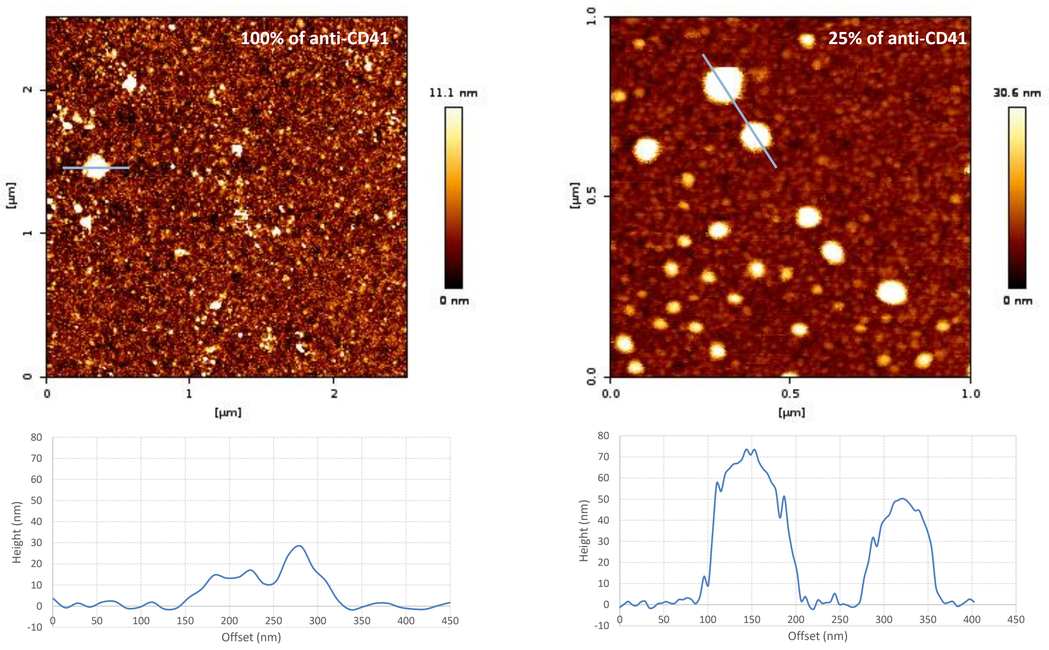
Size distribution diagram of EVs calculated from height and slope images



Size distribution diagram of EVs captured on anti-CD41 spot after the correction of background.

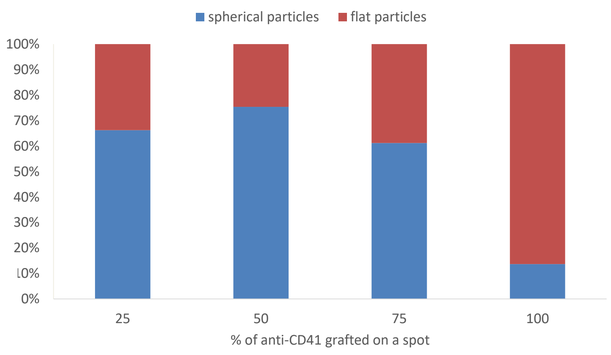
✓ Slope image is less noisy for EVs nanoinvestigation

Influence of specific antibody density on AFM imaging

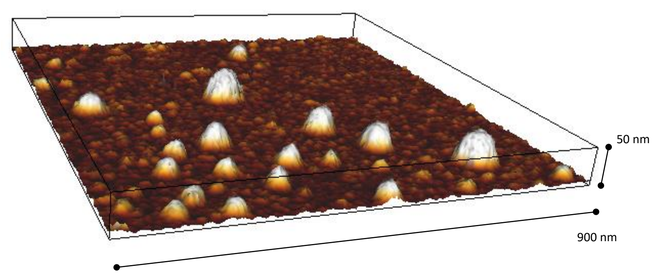


AFM height images and of EVs captured on a spot containing 100% of anti-CD41 (left) and 25% of anti-CD41 (right) with height profiles of selected zones

➢ Dilution of specific antibodies during the grafting step reduces the flattening of the vesicles



Percentage between spherical and flat particles captured at spots with different content of anti-CD41. (The particles with ratio diameter/height > 1.5 are considered as flat and diameter/height ≤ 1.5 are counted as spherical)



3D AFM image of EVs captured on a spot containing 25% of anti-CD41

Conclusions:

On-chip analysis of EVs using the combination of SPR and AFM techniques allows thorough quantitative and qualitative investigation of the sample. We suggest several guidelines regarding AFM analysis in order to limit the deformation of the vesicles on the surface

- Perform the analysis in liquid, avoid drying of the samples, which could lead to particles shrinking and deformation;
- Privilege non-contact mode with low lateral and peak forces (tapping, QI);
- Reduce surface density of antibodies during the specific capture in order to limit the flattening of the particles;
- Analyze simultaneously several parameters (*e.g.* height, stiffness, adhesion force) in order to get more information about the nature of the particles and to discriminate from the background.

Perspectives:

- Investigation of the biointerface and the behavior of the antibodies during the grafting step;
- Comparison of AFM/SPR data with measurements in volume (TRPS, DLS, NTA);
- Optimization of the AFM probe and limiting of tip-induced effects on AFM imaging and analysis.

Reference:
S. Obeid, A. Ceroi, G. Mourey, P. Saas, C. Elie-Caille, W. Boireau. Development of NanoBioAnalytical platform for "on-chip" qualification and quantification of platelet-derived micorpartilces. *Biosensors and Bioelectronics*, **2017**, *93*, 250-259

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