Bacterial adhesion at the naoscale -probing the required cell-surface contact area and role of fibrinogen using a gradient in surface nanotopography

Hulander, M.[1], Valen Rukke, H.[2] and Andersson, M.[1]

[1] Chalmers University of Technology, Sweden, [2] NIOM, Norway

Lately, evidence has shown that bacterial cells are sensitive towards nano-sized surface features and that these may therefore be utilized as a strategy for reducing the adhesion and survival of bacteria on biomaterial surfaces [1]. However, the underlying mechanism(s) for bacterial adhesion on nanostructured material is still largely unknown. We have recently developed a nanostructured surface where the number of nanostructures can be tailored in a gradient fashion along the surface to study these phenomena [2,3]. Here we have used this gradient in a parallel flow chamber to study how the total cell-surface contact area affects the adhesion of *S.epidermidis* to nanostructured surfaces pre-treated in various ways.

Gradients with 40nm sized SiO_2 nanoparticles were prepared by binding the particles to a SAM of APTES (aminopropyl triethoxysliane) on standard microscope glass slides. The surfaces were heat treated for 60min at 400 °C to achieve uniform surface chemistry. These were either used *as is,* or pre-treated with human fibrinogen to evaluate the role of fibrinogen for cell adhesion on nanostructured surfaces. On an additional set of gradients, the space in between the particles was functionalized with NHS-PEG (5 kDa) to create a gradient of "islands" of SiO_2 particles against a non-stick PEG background to function as a control where bacteria could only adhere to the particles. *S. epidermidis* (O.D 1.5) was incubated at room temperature in PBS buffer on the surfaces mounted in a parallel flow chamber and subjected to laminar flow. Cells were stained with acridine orange and images were taken every mm along the gradient using fluorescence microscopy and counted using the software ImageJ. Samples were also viewed in SEM tilted at an angle of 60° to study the interface between the cell and substrate.

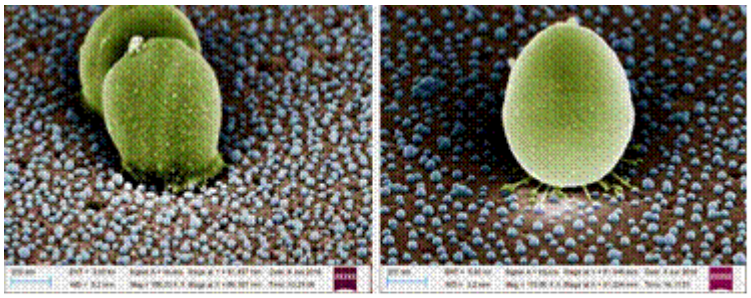

Fig. 1. False color SEM images of S.epidermidis on two different parts of the nanostructured gradient. Note the larger cell-surface interface in the left image.

The effect of nanoparticle coverage for the cell-surface contact of *S. epidermidis* is apparent in the false color SEM images in fig.1. After incubation and subjection to flow, the number of remaining bacteria on the gradients correlated with the number of nanoparticles per m^2 (available surface area). We therefor conclude that adhesion of *S. epidermidis* can be manipulated by changing the cell-substrate area on the nanoscale. Intriguingly, on gradients pre-treated with fibrinogen, the opposite was found, stressing the importance of surface nanotopography for protein mediated adhesion. To further investigate the nature of fibrinogen adsorbed onto nanoparticles we have developed a method using scattering scanning nearfield optical microscopy (sSNOM), an AFM based IR technique using synchrotron light, to probe the secondary structure of single proteins adsorbed on individual nanoparticles for the first time (Fig.2). Results from the flow chamber, SEM, and sSNOM measurements will be presented.

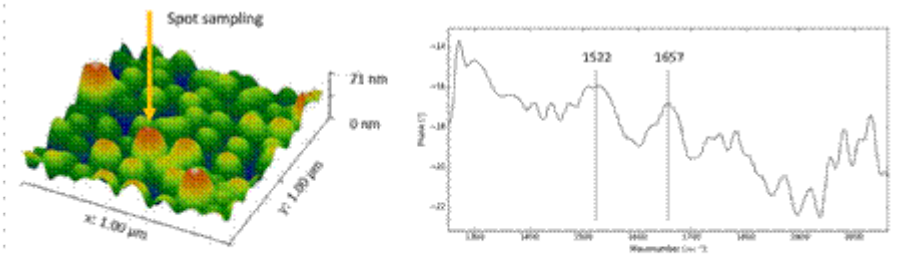

Figure 2. sSNOM measurement of proteins on single nanoparticles. Left: AFM amplitude data with the sampled nanoparticle indicated. Right: Spectrum of the IR signal sampled from the same single nanoparticle with amide I and II bands originating from adsorbed proteins highlighted.

References

1 Rizzello, L., Cingolani, R. & Pompa, P. P. Nanotechnology tools for antibacterial materials. *Nanomedicine* **8**, 807-821, doi:10.2217/nnm.13.63 (2013).

2 Hulander, M. *et al.* Gradients in surface nanotopography used to study platelet adhesion and activation. *Colloids Surf B Biointerfaces* **110**, 261-269, doi:10.1016/j.colsurfb.2013.04.010 (2013).

3 Lundgren, A. *et al.* Gold-Nanoparticle-Assisted Self-Assembly of Chemical Gradients with Tunable Sub-50 nm Molecular Domains. *Particle & Particle Systems Characterization*, doi:10.1002/ppsc.201300154 (2013).