Adhesive heterogeneity of bacterial cell surfaces

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Abstract

Bacterial adhesion is of the utmost importance in the study of environmental bioremediation and the design of materials for medical applications. The understanding of the mechanisms that govern cell-surface interactions must be analysed from the physics point of view in order to obtain quantitative descriptors. [1]

The adhesive properties of *Pseudomonas putida*. (Pse 1), a Gram negative bacteria, has been studied using the scanning force microscope (SFM). Individual cells were imaged and mapped in physiological conditions. (i.e. buffered solution)

The adhesion maps produced by this research work suggest that the Pse 1 surface is heterogeneous. This information will increase our understanding of the mechanisms of biofilm formation.

Figure 1. SFM 3D view of a Pse1 cell.

SFM analysis

The SFM was operated in imaging (x,y,z movement) and force-volume mode, a two dimensional array of force distance measurements (z movement) over an area to display images of force and height variations.

Topographic images of individual Pse1 cells were acquired under a buffer (MOPS* 20 mM, pH 7.4) using a Molecular Force Probe 3D (Asylum Research) scanning force microscope, operated in intermittent contact mode. This mode ensures minimum interaction with the delicate bacterial surface. Details of the cell surfaces and appendages can be observed.

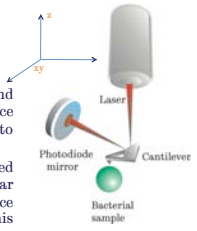
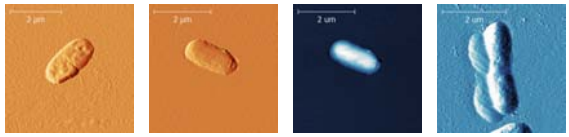


Figure 2. SFM scanning system



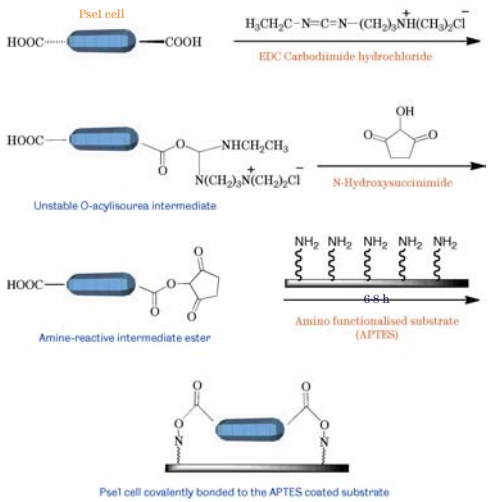
Amplitude image | Amplitude image | Height image | Amplitude image

Figure 3. SFM images of Pse 1 cells

*MOPS: 3-(N-morpholino)propanesulfonic acid

Sample preparation

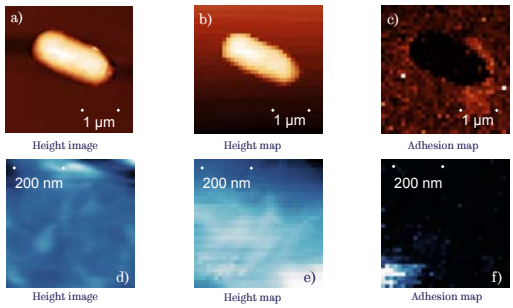
To examine bacteria under liquid, the cells need to be firmly attached to the substrate. One commonly used technique is to employ covalent bonding between bacterial cells and molecules attached to a slide. For several Gram negative species the crosslinking reaction between EDC (1-ethyl-3-(3-dimethylaminopropyl)) carbodiimide hydrochloride and N-hydroxysuccinimide with an APTES (3-aminopropyltrietoxy silane) coated substrate, has been used.[2] (Scheme 1)



Scheme 1. Chemical reaction of bacterial immobilisation.

Mapping

Height and adhesion maps of were acquired using force-volume mode. Images 4a-c depict the entire cell, whereas images 4d-f correspond to a section within the same cell. Figure 4f reveals heterogeneous adhesive measurements.



Height image | Height map | Adhesion map

Height image | Height map | Adhesion map

Figure 4. SFM height images (a,d), height maps (b,e) and adhesion maps (c,f) of a Pse 1 cell.

Conclusions

Pse1 cells can be effectively immobilised using covalent bonding and analysed using SFM. High resolution images were obtained in a physiological environment, as well as height and adhesion maps. The latter reveal a heterogeneous adhesive behaviour. The acquisition of adhesion maps in variable conditions will give us a deeper insight into the nature of *Pseudomonas* biofilm formation

Further information

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References: [1] Andrews, J.S., Rolfe, S.A., Huang, W.E., Scholes, J.D. and Banwart, S.A. *Environ. Microbiol.*, **12**, 2496 (2010), [2] Liu, Y and Camesano T A. (2008). Immobilizing Bacteria for Atomic Force Microscopy Imaging or Force Measurements in Liquids. In: Camesano T A and Mello C Microbial Surfaces. OUP USA: ACS Symposium Series. 163-188.