Bacterial biofilms investigated by atomic force microscopy and electrochemistry

Bacterial biofilms are aggregates of microorganisms in which cells adhere to each other and adhere to a solid surface or an animal host cavity. Bacterial biofilms play important roles in human life, and cause serious harm for human society and huge economic losses. The complex composition of bacterial biofilms with EPS (Extracellular polymeric substances) includes proteins, polysaccharides, extracellular DNA (e-DNA), peptidoglycans, lipids and phospholipids. These substances play an important role in the initial adhesion of bacteria to the surface and maintenance of the biofilm structure. In my thesis, Atomic Force Microscopy (AFM) and electrochemistry have been applied to investigate three pathogenic medically important bacterial biofilms, i.e. *Pseudomonas aeruginosa* (cystic fibrosis pneumonia), *Staphylococcus epidermidis* (contamination of surgical catheters and indwelling equipment) and *Streptococcus mutans* (dental caries).

AFM was used to investigate the adhesion force on single live cell surfaces. Four different strains of *Staphylococcus epidermidis* in liquid aqueous environments were addressed. These strains were selected because of their special surface proteins related with the initial attachment on the surface. High-resolution AFM imaging showed no detectable differences among the four strains. Adhesion maps using hydrophobically modified tips compared with bare hydrophilic silicon nitride tips also showed small differences only. This indicates that hydrophobic effects are not the primary driving forces towards adhesion. Two chemical inhibitor compounds were found to have strong effects on the adhesion between the bare tips and the bacteria.

Secondly, AFM and electrochemistry were combined to study bacterial biofilm formation on Au(111)-surfaces, to determine the surface charge and growth pattern of *Streptococcus mutans* biofilms. Five redox probes were chosen for cyclic voltammetry, i.e. positively, [Ru(NH₃)₆]³⁺/²⁺, [Co(phen)₃]³⁺/²⁺ and [Co(terpy)₂]³⁺/²⁺ (phen = 1,10-phenanthroline; terpy = 2,2',2''-terpyridine) and negatively charged, [Fe(CN)₆]³⁻/⁴⁻, [IrCl₆]³⁻/⁴⁻. The inhibition and voltammetric patterns showed that S. mutans biofilm are negatively charged. Addition of DNAase suggests that the negative charges to a large extent originate from DNA excreted by the biofilm.

Thirdly, AFM and electrochemistry were combined to study *Streptococcus mutans* biofilm formation on bare Au(111) and Au(111) modified by self assembled molecular monolayers (SAMs) of thiol-based molecules. Four SAM molecules were chosen for reductive desorption (RD). Two are long straight-chain thiols with either a hydrophobic or a hydrophilic terminal group, i.e. hexadecanethiol, HS(CH₂)₁₅CH₃ and mercaptopentadecanoic acid, HS(CH₂)₁₅COOH. Two others were the short rigid linker molecules L-cysteine and cysteamine. Strong RD peaks were obtained for all the four molecules both in PBS buffer at pH 7.4 and in 0.1M NaOH solution. Both AFM images and the electrochemical data show further, that the biofilms are bound more strongly to the hydrophobic surface than to the hydrophilic surfaces.

Finally, AFM was used to study two other kinds of bacteria, *Pseudomonas aeruginosa* and *Pseudomonas putida*, and their relationship with EPS. Different mutant strains were applied to investigate the roles of Pel and Psl polysaccharides and type IV pili during *P. aeruginosa* biofilm development. This study suggests that polysaccharides and e-DNA contribute to the biofilm development. Protein clusters were observed during P. putida biofilm formation, but we need further investigation to identify or distinguish the surface protein Lap A and Lap F. The combination of AFM and electrochemistry is a new approach to understand the bacterial biofilm/ medium / bare or modified Au(111) interface. Future efforts aim at observing the live bacterial biofilms with high resolution and controlling the biofilm formation on SAM-modified Au(111)-surfaces by the combination of AFM and electrochemical characterization.

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