Macroscopic and microscopic approaches toward bacterial adhesion

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Application of physico-chemical models to explain microbial adhesion to surfaces has been successful for a limited number of strains and species (Chapter 1), owing to the macroscopic nature of the input data (i.e. zeta potentials and contact angles). Microorganisms have, from a physico-chemical point of view, generally been considered to be similar to inert polystyrene particles. However, microorganisms are not smooth particles and, in contrast to polystyrene particles, carry long, usually very thin surface structures protruding from the cell surface and radiating outwards into the surrounding liquid. Yet, the function of these surface structures in specific adhesion processes to different substratum surfaces as well as their influence on overall physico-chemical cell surface characteristics, remains to be identified for most bacterial strains. Therefore, relevant physico-chemical measurements on microbial cell surfaces require a microscopic resolution that can not be accomplished with most currently employed methods.

The introduction of the Atomic force microscope (AFM) and its application to biological surfaces has opened a new avenue to obtain microscopic, physico-chemical properties of bacterial cell surfaces. With a long-term goal of developing
a new theory for bacterial adhesion which will account for more complex interactions (e.g. charge heterogeneity, macromolecular bridging) that are not accounted for in DLVO theory, we initiated this thesis. The aim of this thesis was to reach a microscopic characterization of physico-chemical properties of bacterial cell surfaces as well as to find out if and how microscopic properties could be amalgamated into macroscopic cell surface properties and related to macroscopic bacterial adhesion on solid substrata.

A collection of nine Streptococcus mitis strains had been macroscopically characterized with regard to their cell surface hydrophobicities by water contact angles, and its surface chemical composition using X-ray photoelectron spectroscopy. In order to complete their macroscopic surface characterization, the electrophoretic softness and fixed charge density in the polyelectrolyte layer of S. mitis strains, usually carrying long sparsely distributed fibrils, were determined by the soft particle analysis using measured electrophoretic mobilities as a function of the ionic strength (Chapter 2). In general, S. mitis cell surfaces are electrophoretically soft with negative fixed charged density. Further, a comparison with surfaces of other bacterial strains that are reported to be soft indicates that the Ohshima theory does not provide information on the surface morphology causing the softness. The most likely reason is that the electro-osmotic flow occurs only in the very outer region of thick extracellular surface layers. Nevertheless, determining the surface softness was essential for proper characterization of the cell surface electrostatics.

For AFM experiments, bacterial cells need to be firmly anchored to a substratum surface in order to withstand the friction forces from the silicon nitride tip. In order to probe physico-chemical properties of bacterial cell surfaces under physiological conditions, it is required that immobilization does not affect the chemical and structural integrity of the cell surface. Different strategies for the immobilization of bacteria have been described in literature. Chapter 3 compares AFM interaction forces obtained between Klebsiella terrigena and silicon nitride for three commonly used immobilization methods, i.e. mechanical trapping of bacteria in membrane filters, physical adsorption of negatively charged bacteria to a positively charged surface and glutaraldehyde fixation of bacteria to the tip of the AFM. It was shown that different sample preparation techniques give rise to dissimilar interaction forces. Indeed, physical adsorption of bacterial cells on modified substrata may promote structural rearrangements in bacterial cell surface structures while glutaraldehyde treatment is thought to induce physico-chemical and mechanical changes on bacterial cell surfaces properties. In general, mechanical trapping of single bacterial cells in filters appears to be the most reliable method for immobilization.

After having established the most suitable bacterial immobilization method for AFM, a detailed analysis of the interaction forces between a silicon nitride AFM tip and the surface of the nine different oral bacterial S. mitis strains, was carried out. Chapter 4 presents a first attempt to correlate microscopic and macroscopic bacterial cell surface properties. Interestingly, microscopic features of force-
distance curves could be amalgamated in such a way, that relations between microscopic cell surface properties and macroscopic cell surface properties were obtained, even though these relations were not fully understood.

In **Chapter 5**, we attempted to correlate microscopic adhesion of *S. mitis* strains to the negatively charged, hydrophilic, silicon nitride tip of an AFM with macroscopic adhesion of the isolates to a negatively charged, hydrophilic glass in a parallel plate flow chamber. The repulsive force probed by AFM upon approach of the tip to a bacterial cell surface was found to correspond with an activation barrier, governing initial, macroscopic adhesion of the organisms to the glass surface. Moreover, maximum distances at which attractive forces are probed by the AFM upon retraction of the tip related with the area blocked by an adhering bacterium, *i.e.* the distance kept between adhering bacteria. Bacterial desorption could not be related with adhesive forces as probed by the AFM, possible due to the distinct nature of the desorption process occurring in the parallel plate flow chamber and the forced detachment in AFM.

Microbial desorption had also been studied in situ in controlled flow devices as a function of the organisms resident time on the surface (Meinders et al., 1994). It appeared that desorption of *Streptococcus thermophilus* decreased strongly within approximately 50 s after initial adhesion due to bond aging. In **Chapter 6**, bond aging between the *S. thermophilus* cell surface and the silicon nitride tip of an AFM was corroborated microscopically and related to the macroscopic, residence time-dependent desorption of the organism under flow. AFM indicated bond strengthening between the tip and the cell surface within 100 s of contact, which is in the same order of magnitude as bond aging inferred from residence-time dependent desorption. Additionally, comparison of the interaction energies derived from AFM and macroscopic desorption, seems to indicate that bond strengthening arises as a result of multiple attachments of extracellular polymeric substances to a substratum surface.

Bacterial surface hydrophobicity, generally measured by placing water droplets on carefully prepared and dried microbial lawns, is commonly accepted as influential on bacterial interaction with their environment. However, contact angles measured with liquid droplets on a microbial lawn are essentially representative of a fuzzy coat of cellular surface material, collapsed into a lawn. Therewith results are useful to interpret the long-range interactions between an organism and a substratum surface, but not necessarily for the interpretation of short-range interactions, which may be dominated by structural and chemical cell surface heterogeneities. In **Chapter 7**, the surfaces of *L. acidophilus* ATCC4356 and *L. casei* ATCC393 with and without SLP respectively, have been probed with regard to their interaction forces with chemically functionalized AFM tips, *i.e.* terminated with hydrophobic (CH$_3$) and hydrophilic (OH) groups at low and high ionic strength solutions. The macroscopic cell surface hydrophobicity of the two strains was also assessed by contact angle measurements using the two ionic strength solutions investigated. In general, high interaction forces with a hydrophilic tip were found to coincide with low contact angles, whereas a cell surface with high
contact angle showed the strongest interaction with a hydrophobic tip. In addition, both strains reversed their hydrophobic nature upon increasing the ionic strength from 10 to 100 mM. It is interesting, that the dynamic behavior of the cell surface hydrophobicity of the lactobacilli was not only measurable macroscopically by contact angles on bacterial lawns, but also by AFM at a more microscopic level. This dynamic behavior of bacterial cell surfaces upon changes in ionic strength offers to \textit{L. acidophilus} ATCC4356 and \textit{L. casei} ATCC393 a versatile mechanism to adhere to hydrophobic and hydrophilic surfaces in low and high ionic strength solutions, respectively.

It is commonly found that adhesion of lactobacilli to solid substrata does not proceed according to expectations based on cell surface hydrophobicity. In \textbf{Chapter 8} the role of cell surface hydrophobicity of the two lactobacillus strains with and without a SLP layer has been investigated with regard to their adhesion to hydrophobically or hydrophilically functionalized glass surfaces under well-defined flow conditions and in low and high ionic strength suspensions. Similarly, the interaction of the lactobacilli with similarly functionalized AFM tips was measured. In a low ionic strength suspension, both lactobacillus strains show higher initial deposition rates to hydrophobic glass than to hydrophilic glass, whereas in a high ionic strength suspension no clear influence of cell surface hydrophobicity on adhesion is observed. Independent of ionic strength, however, AFM detects stronger interaction forces when both bacteria and tip are hydrophobic or hydrophilic than when bacteria and tip have opposite hydrophobicities. These strong adhesion forces are not reflected in macroscopic observations on bacterial adhesion which suggest that the force applied when a bacterium comes into contact with a substratum influences its adhesion mode. In addition, the distance dependence of the total Gibbs energy of interaction could only be qualitatively correlated with bacterial deposition and desorption in the parallel plate flow chamber whereas AFM measurements failed to detect DLVO forces upon approach of the functionalized tips to the bacterial cell surfaces.

In the \textbf{General Discussion}, the main conclusions drawn in the thesis are further discussed, being:

(a) approach lines of force-distance curves correspond with the rate of initial, macroscopic adhesion of the organisms to a substratum,

(b) the maximum distance over which attractive forces are probed by AFM upon retraction of the tip are influential on the area blocked by an adhering bacterium on a substratum and

(c) bacterial desorption from solid substrata does not show a relation with adhesive forces as probed by AFM unless the contact time between bacterium and substratum is taken into account.

In addition, some of the limitations of the system regarding accurate interpretation of force-distance curves as well as future perspectives are presented.