Direct observation of particle deposition in a parallel plate flow cell; Application to oral streptococci and polystyrene particles

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SUMMARY

Bacterial deposition is the first important step in the bacterial colonization of host surfaces leading to for instance dental caries and many infectuous diseases. Deposition is governed by long range interaction forces e.g electrostatic, van der Waals and steric interactions. It is the first step towards adhesion which is governed by much shorter range forces e.g. hydrophobic, hydrogen and chemical bonds. A better knowledge of the deposition process may contribute to the prevention of bacterial infection and caries.

Previous studies on bacterial adhesion showed a relation between the number of adhering bacteria in an equilibrium situation and the surface free energy of both the bacteria and the solid substrata. However, the influence of the hydrodynamic conditions during deposition as well as the combined influence of van der Waals and electrostatic interactions on bacterial deposition is relatively unknown. This is the main objective of the present investigation which is summarized below.

In chapter 1 the three aims of this thesis are presented. The first aim is to construct a flow cell system allowing direct observation of bacterial deposition. The second aim is to solve the convective diffusion equation for this flow system, which enables the theoretical prediction of the deposition rate of colloidal particles from flowing suspensions. The third aim is to measure bacterial deposition and to interpret the results in terms of a theoretical model. Furthermore it is pointed out that in order to allow theoretical predictions and interpretations it is useful to carry out experiments with polystyrene particles as a model system. For the same reason polymers, glass and mica were chosen as substratum surfaces. Because the convective diffusion theory mentioned does only take into account colloidal interactions, the investigations were mainly limited to the influence of these types of interactions on deposition.

Chapter 2 gives a literature survey of various types of experimental systems employed for studying bacterial and cellular adhesion to solid substrata. It is argued that it is advantageous to employ flow sytems rather than static systems because fixation and staining procedures can often be avoided and hydrodynamic conditions can be defined accurately. Furthermore particle transport to a solid substratum can be calculated theoretically from the convective diffusion equation. An analytical solution of the convective diffusion equation is presented for a parallel plate flow cell neglecting particle/substratum interactions. An approximate analytical solution taking into account these interactions is described, together with some results of numerical procedures. The interaction potentials involved in these calculations are the van der Waals and electrical double layer interaction potentials, which are described in the last section of this

chapter.

In chapter 3 a description is given of a parallel plate flow cell system and illumination facilities employed in this study enabling direct observation of bacterial and particle deposition. In addition a method for determining the number of adhering microorganisms by using automated image analysis is presented. Deposited bacteria are observed by a video camera mounted on a phase contrast microscope and coupled to an image analysing computer. From images, digitized at various time intervals after the onset of the experiment, object area histograms are generated from which numbers of adhering single bacteria, doublets and higher multiple aggregates can be doublets calculated using a manual calibration performed on a limited number of images stored during the experiment.

In chapter 4 experimental deposition data are presented of several bacterial strains and polystyrene particles on glass. Three bacterial strains with known surface free energy and zeta potentials (*Streptococcus mutans* NS, *S. sanguis* CH3 and *S. mitis* BMS) and two strains with well defined surface appendages (*S. salivarius* HB and *S. salivarius* HB-C12) have been used. The bacterial and particle deposition rates have been measured on the top and the bottom plate of the cell and were interpreted on basis of the solution of the convective-diffusion equation neglecting particle/substratum interactions. A marked sedimentation has been observed. It is argued on basis of the height of potential barriers calculated between particle and substratum and a comparison with polystyrene particle deposition rates that bacterial cell surface appendages assist deposition. Finally it is shown that the decrease of the deposition rate as a function of time can be described on basis of a blocking effect, which is constant in time.

In chapter 5 a new method is introduced for solving the convective diffusion equation for a parallel plate flow cell taking into account repulsive particle/substratum interactions. This method combines the Surface Force Boundary Layer Approximation with a numerical method for solving the exact convective diffusion equations. The results of this method are related to deposition on polymethylmethacrylate (PMMA) for two types of polystyrene particles at different flow rates and ionic strengths. The experimental data indicated that the theory describes deposition phenomena correctly. Observed discrepancies between the theory and experiments are ascribed to substratum and particle heterogeneity and the hairiness of the polystyrene particles.

The influence of the substratum surface free energy and zeta potential on the deposition rate of polystyrene particles is discussed in chapter 6. The polystyrene particle deposition rate was measured on four different types of substrata: mica, glass (both possessing high surface free energy), polymethylmethacrylate (PMMA) and fluorethylenepropylene (FEP) (possessing intermediate and extremely low surface free energies, respectively) at different ionic strengths. It was observed, that the discrepancy between the theoretically predicted and experimentally determined critical ionic strength

for deposition, at which a transition between fast deposition (diffusion and convection controlled) and low deposition (interaction controlled) occurs, is dependent on the substratum surface free energy. The deposition rate on FEP was unexpectedly high at low ionic strengths, whereas on mica and glass the experimentally determined critical ionic strength coincided with theoretical predictions. It is suggested that this discrepancy originates from an attractive long range hydrophobic interaction not accounted for in the classical colloidal stability theories (DLVO theory). The attractive force is possibly due to a perturbation of the vicinal water structure by a hydrophobic surface.

In chapter 7 the distribution of deposited polystyrene particles on FEP, PMMA and mica is discussed on the basis of radial and angular pair distribution functions. The pair distribution functions indicate the screening distance over which deposited particles prevent flowing particles to deposit (radial pair distribution functions) and the occurrence of preferential angular orientations of deposited particles relative to the flow direction (angular pair distribution functions). From the relation between distribution functions of deposited particles and computer simulated random distributions it was concluded that the observed unfavourable deposition near already deposited particles is due to hydrodynamic interactions. Angular pair distribution functions revealed that particle deposition is most unfavourable in narrow angular sections in front and behind deposited particles which is ascribed to repulsive interactions between deposited and flowing particles. These screening effects as expressed by both pair distribution functions, determined the spatial arrangement especially on PMMA which is explained by a more effective immobilisation on PMMA than on FEP or mica. Finally it is mentioned that a distinction should be made between the screening distance and the equilibrium interparticle distance. Large differences were measured between both parameters indicating that the number of deposited particles in equilibrium is mainly determined by collector surface heterogeneity and desorption.

In chapter 8 the deposition is studied of two oral streptococcal strains with well defined surface appendages on four different substrata at various buffer concentrations. *S. salivarius* HB possessed extended cell surface appendages, whereas *S. salivarius* HB-C12 was a completely bold variant. Both strains showed a fast deposition on FEP even at low ionic strengths, which was not predicted by theoretical calculations on basis of the DLVO theory. These experimental results which could only be explained by the introduction of an additional attractive hydrophobic interaction, supported the conclusions arrived at in chapter 6 for polystyrene particle deposition. Only *S. salivarius* HB showed high deposition rates on the other substratum materials employed: PMMA, glass and mica, probably due to the hydrophobic character of their cell surface appendages, absent on the variant strain *S. salivarius* HB-C12. These appendages could give rise to hydrophobic bonds with the

substratum surface or induce a local decrease of the potential barrier height due to their small radius.

In chapter 9 the spatial arrangement of deposited bacteria on glass is studied on basis of radial and angular pair distribution functions. For *S. salivarius* HB a small screening distance was measured as compared to *S. mutans* NS. Also high first order maxima were observed for *S. salivarius* HB which were practically absent in the distribution functions of *S. mutans* NS. In addition *S. salivarius* HB showed an apparent preference for deposition parallel to the flow direction on both sides of a deposited bacterium. This characteristic behaviour of *S. salivarius* HB was explained on basis of cooperative effects involved in its deposition originating from attractive interactions between extended cell surface structures. Consequently, the completely different spatial arrangement of *S. mutans* NS was ascribed to the absence of such extended cell surface structures.

Most of the work described in this thesis is based on polystyrene particles and model substrata. Yet the techniques and theories developed can (and should) be used in the future toward studying bacterial adhesion. The technique for the enumeration of deposited particles in a flow cell is probably unique and can also easily be extended for the direct measurement of desorption.

Furthermore the method of analysing the spatial arrangement by means of pair distribution functions can be an important tool for studying cooperativity and noncooperativity which are thought to be important factors in the formation of dental plaque.