Computer aided research in microbial and particle adhesion

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CHAPTER 8

General discussion

The aim of this research was firstly to develop better quantitative image analysis methods for the study of microbial adhesion, also applicable for substrata yielding bad quality images, and secondly, to measure with these methods the particle deposition for various substrata. This should yield a complete description of the adhesion kinetics involving the main macroscopic aspects of the process. An onset was given earlier, by the pioneering research of Sjollema [1], and later on by Meinders [2].

Because part of this thesis is about computer methods, and part about the deposition measurements carried out with them, the title combining both aspects is chosen as “Computer aided research on microbial and particle deposition”. Accordingly, attention is given to the aid that computers provide, and to the positive and negative implications of the use of computers in this line of research.

Firstly, computers can relieve the experimenter of the simple but elaborate task of particle enumeration in microscope images. Even if counting ‘by hand’ is preferred the computer can serve as digital video recorder for grabbing of images at set times. Furthermore, deposition research has received new impulses because image analyses that were previously impossible to carry out - or only at the expense of vast efforts - are now at hand. Examples include the determination of adsorption and desorption rates, particle residence times, desorption probabilities, and radial distribution functions [3, 4, 5]. Also, new theories and models can be developed with the help of computer simulations [6, 7], such as carried out in Chapter 5 of this thesis. Computer simulations, as well as numerical methods, are especially well suited for the complex circumstances where analytical methods fail, such as in theoretical calculations involving convection, diffusion, and electrochemical interactions. Furthermore, they provide for a virtual method to predict the results of changing an input parameter. Expectations are that future increases in computer power will make simulations and numerical calculations possible which encompass most aspects influencing the deposition process, such as interactions between particles and substrata, hydrodynamic factors, Brownian motion, and bacterial growth factors.

Summarizing, computers make deposition experiments less time and labor expensive and allow for more complex and even virtual experiments, which has an important side effect that is often overlooked. The research worker can now play around more with the data and experimental circumstances because calculations are faster, relations are more easily verified, and data can be presented in different ways. As a result, the attentive experimenter has a greater chance of running into unexpected discoveries which provide an onset for extended research. This is also known as serendipity, which always has been a most important factor in the progress of science.

The use of computers also has disadvantages. Some can be regarded as temporary obstacles to be overcome by future technical developments, while others are inherent to the automation itself. An example of a temporary nuisance is the low processing speed of the artificial neural network in comparison with other methods used for enumeration and position detection of
adhering particles, as presented in Chapter 4 of this thesis. Artificial neural networks are at least as accurate as other methods, but are computationally expensive, especially if they are implemented with an extra neuron layer for more complex analysis. If however these calculations are carried out by specially designed hardware, or by faster processors commonly available in the future, neural networks will certainly become the method of choice for pattern recognition purposes. The only limitation will then be the accuracy of pattern recognition, which is determined mainly by how well the human experimenter feeds the network with relevant training examples.

Another, more general remark about automation of research, is that by some experimenters computer results are used without checking or being aware of error margins. Equally counterproductive is when experimenters excessively distrust computer results. An example is the computer program developed at this laboratory for deposition experiments, which some users merely regard as a video image recorder, and rather laboriously enumerate particles ‘by hand’ even if the images are well suited for automatic processing. Their argument that hand counting is more accurate was disproved in Chapter 4, in which hand countings were compared with computer enumerations for standard quality images, yielding an error margin of about 5% for both methods. Therefore, psychological factors play a role here, which are observed more often in relation with computer use, such as the reluctance to hand over the control to this machine, the overestimation of one’s capacities, and some computerphobia. Often these factors originate from unawareness of what the computer program does or how it must be used, or from reluctance to learn about it. Thus, during the development of this program it became clear that it should be kept simple and straightforward to use, while the users should be instructed thoroughly to become aware of the capabilities and limitations. Moreover, since a good program is a frequently used one, it was simplified at the expense of extended possibilities and now the revised version is used more.

A limitation of computer simulations and numerical calculations, is that they will never be able to fully replace real experiments, even with a zillion times larger computing power. This is because particle deposition is complex and chaotic, involving many aspects that may lead to instabilities such as convection, thermal interactions, Brownian movement, and other non-linearities. The beginning conditions and the laws of nature governing the process would have to be known infinitely precise to make reliable long-term predictions, which is of course impossible. Chaotic systems can exhibit vast changes in output behaviour upon seemingly insignificant changes of beginning conditions, which is also known as ‘the butterfly effect’ [8]. An everyday example is the weather that presently has a forecasting horizon of approximately five days, which is a meager gain of only two days since the computer stone age of some thirty years ago. Although this is somewhat disappointing, simulations of chaotic systems may on the other hand reveal very simple causes for complex and previously misunderstood phenomena, such as fractal structures.

In Chapters 5, 6, and 7 the focus moves towards particle deposition research. Several factors of influence on adsorption and desorption were investigated, a numerical model was presented for the deposition kinetics, a computer simulation was carried out, forced desorption by an air bubble was investigated, as well as redeposition of particles thereafter. Several observations in all these chapters cannot be explained by presently available theories and all point towards a common factor, which is surface heterogeneity. This especially shows for low
solution ionic strengths where energetic circumstances are less favorable for adhesion. Present theories and calculational models all assume homogeneous and smooth surfaces, and as a result theoretical predictions are incomplete. In Chapter 5 for example, the initial deposition rate was expected to rise quickly from zero to a maximum value above a certain ionic strength, while on the other hand measurements showed a smooth and gradual rise upon increasing ionic strength. A similar curiosity was observed by comparing the surface coverage at different ionic strengths. The distribution of particles on the substratum yielded the same minimum possible distance between particles for both ionic strengths, which was about one particle diameter. For high ionic strengths, at the end of flow experiments when the surface coverage had reached a steady state, the substratum was covered densely and in agreement with this distance. However for low ionic strengths, the surface was much less densely covered. Clusters of particles were adhering side to side and covering patches of the substratum surface, while also large empty patches were observed. These two observations suggested that the applied substratum surface must be inhomogeneous with respect to adhesion affinity.

This hypothesis is supported by results presented in Chapter 6, where passage of an air bubble did not remove all adhering particles, suggesting a possible inequality in adhesion force. However, this may also be explained by the so called ‘bond-aging’ effect [9], which refers to the decrease in desorption probability with particle residence time. Supposedly, thermal vibrations and hydrodynamic changes in the fluid layer between particle and substratum cause a particle to slowly settle into a more favorable energetic situation. Therefore it should be investigated if the removed particles of Chapter 6 were the relatively shorter adhering ones. If not, surface heterogeneity is the potential explanation here too. A first direct proof of surface heterogeneity was found in Chapter 7, where the affinity for adsorption was shown to be more than 20 times larger at positions that had previously been occupied. That the effect is not due to changes in the substratum caused by the previously adhering particles, or ‘footsteps’ left behind, but rather is a preexisting property of the substratum, was proven by the reverse observation that the high affinity sites found after air bubble passage were also first occupied at the beginning of the experiment.

Most of the experiments leading to these observations were carried out on glass substrata and with polystyrene particles, and therefore deducted theories may not be extrapolated to other materials. To allow such extrapolations, similar investigations should be carried out for other combinations of biomaterials and microorganisms. However, the previously mentioned contradictions between theory and measurements imply that existing theories must be extended to include surface heterogeneity as a factor. Therefore the concept ‘blocked area’ should be revised, as this is presently one single parameter that has been used incorrectly for two different observables. One observable is the area occupied by one particle within which no other particle can adhere, following from the minimum possible distance between particles. The other is the average area occupied by one particle, following from the substratum surface coverage. It was already mentioned that these two observables can be very different, especially at low ionic strengths. A good measure for surface heterogeneity would therefore be the ratio between these two observables.

Furthermore, macroscopic parameters alone appear to be insufficient in covering all aspects of the adhesion process. Small scale effects ranging down to the nanometer size of ions and molecules may play an important role too, for example in the explanation of phenomena
such as surface heterogeneity. Using the parallel plate flow chamber setup armed with new tools for image processing, similar experiments as carried out in Chapter 7 may give an onset for such research. Later are to follow experiments with precision instruments like the atomic force microscope (AFM). For example, an AFM carrying a polystyrene particle or microorganism fixed to the probe could be used to scan within a small area of some 100 nm around a high affinity site found in a preceding redeposition experiment. This might yield an electrostatic factor as a cause for high affinity sites, like ions buried in the substratum surface.

Apart from future research on aspects governing adsorption, additional research is required on desorption aspects as well, to discover why particles desorb spontaneously and how external factors may promote desorption, to investigate the role of thermodynamical and electrochemical factors, of changes in the substratum or solution, of hydrodynamic effects, or interactions of adhering particles with flowing particles. Some of this work is presently carried out in this laboratory. The effect of collisions between flowing and adhering particles on desorption was suggested by Meinders [2], but was not followed up by experiments yet to prove such an effect. For such an experiment, images should be recorded from the flow chamber setup with high speed and high image resolution. Subsequent image processing methods, such as presented in Chapter 2 and Chapter 3, will then yield the tracks of the particles flowing by, whereafter they should be analysed in combination with observed desorption events to prove a potential connection.

Summarizing, several new techniques have been forwarded in this thesis. One is the use of neural networks in microscopic image analysis, which will become the method of choice as computing power increases and research moves towards biomaterials like turbid biopolymers such as silicone rubber, and to plastics and metal. Methods for particle tracking and exact position determination were presented and will be useful when the focus moves from the ensemble to the behaviour of individual particles. The macroscopic approach of particle deposition research has yielded feasible theories and models, which were extended further but also need additional refining to encompass surface heterogeneity as a factor. Stochastical phenomena such as heterogeneities are hard to study by analytical methods alone, and therefore experiments, numerical methods, and simulations will lead the way here such as proposed in Chapter 5.

Eventually, fundamental research on microorganisms and clinical applications of biomaterials must be scaled down to the level of molecules and atoms. The parallel plate flow chamber and polystyrene particle as a model will remain important in the study of macroscopic and clinically relevant effects. In combination with more specific techniques and instruments like the AFM several phenomena should be investigated in more detail, such as forced particle detachment, redeposition probability, and other phenomena that may ultimately be important in the prevention of biomaterial implants infections.

REFERENCES


