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Null Ellipsometry and Protein Adsorption to Model Biomaterials

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Akademisk avhandling

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When implants are inserted into the human body cascades of events become started that will determine the outcome of wound healing and ultimately the success of the implantation. The events start with the adsorption of small molecules, and proteins that may be activated (enzymes) or are able to activate cells of the immune defense and the healing process. In the biomaterials research that is conducted in our group we often ask two questions: "How much?" and "What?" proteins adsorb to a specific surface after incubation in serum or plasma. In the first two papers in this thesis I studied how well we are able to determine the answer to the first question. In the latter two works I tried to answer both questions for two model biomaterial surfaces: oligo(ethylene glycol) terminated self assembled monolayers on gold and chitosan coated silicon.

In many null ellipsometric studies the protein film refractive, N_{film}, is assumed to be close to 1.5. In the first paper we analyzed if the assumption of N_{film} = 1.465 is satisfactory for the determination of the surface mass density of a submonolayer thin protein film. Human serum albumin (HSA) was labeled with ^{125}I and mixed with non-labeled HSA, and hydrophobic and hydrophilic silicon pieces were incubated in the solutions. The surface mass densities on all pieces determined by both ellipsometry and gamma counter measurements, and was pair-wise compared. The above assumption regarding the value of N_{film} for the agreement between the methods was satisfactory, although precautions have to be made not to overestimate the surface mass density when studying radiolabelled proteins, especially at rough surfaces.

Are the assumptions made in Paper I also true for up to 100 nm thick protein films or do we have to use a different protein film refractive index and do the ellipsometric model still hold? Human serum albumin (HSA) and polyclonal anti-HSA were labeled with ^{125}I and mixed with

unlabelled proteins. Hydrophobic silicon pieces were alternatingely incubated in the two protein solutions. Again the surface mass density was quantified with null ellipsometry and a gamma counter and the methods compared. The thickest protein layers were also gently scratched and the thickness measured by AFM. It appeared that a protein film refractive index $N_{film} = 1.5$ was a good choice for the determination of the protein film thickness. However, in order to obtain a good methods agreement for the adsorbed mass density a linear correction term was needed in the Cuypers surface mass density formula for ellipsometry. The physical interpretation of the correction term is presently unclear.

Self assembled monolayers (SAMs) containing oligo(ethyleneglycol) end groups (OEG) have been successfully used to minimize protein adsorption from single protein solutions. We investigated the protein resistance in a fibrinogen solution, serum and plasma of OEG-SAMs with an increasing number of OEG units and with different end groups. It turned out that the adsorbed amounts after 10 minutes of plasma incubation and 15 minutes of fibrinogen incubation decreased with an increasing number of EG units. In serum, the total deposition and subsequent deposition of antibodies towards complement proteins (C3c, C3d and properdin) did not depend on the number of EG units. In summary, the investigated OEG-SAMs were not protein resistant in complex solutions, although the adsorbed amounts varied with the number of EG units and the terminal chemical group. Complement deposition was observed at OEG surfaces.

For the last 50 odd years different polysaccharides, such as heparin and cellulose have been used for clinical applications and in recent years also chitin and its deacetylated form chitosan have gained increasing attention as potential biomaterials. Previous studies on complement activation by chitosan derivatives have focused on the soluble complement factors and not the surface bound ones that may be important for the binding of cells to surfaces. In our study about 10 nm thick chitosan films were incubated in plasma or serum and subsequently in polyclonal antibody solutions. The films did not activate complement and the intrinsic pathway of coagulation although fibrinogen was detectable after plasma incubations. When the chitosan film was acetylated it became a strong alternative complement pathway activator in serum and fibrinogen was then no longer antibody detectable after plasma incubations.

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