Influence of surface charge on the adorption of proteins on titanium

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Summary

When an implant is inserted in the body, its surface interacts with the biological environment of the body. Proteins adsorb within seconds on the surface of the biomaterial, followed by cells interacting with the already adsorbed proteins rather than with the actual implant material itself. The latter cell response is strongly influenced by the adsorbed proteins and their conformations. The surface charge is considered to be one of the main physical factors that influences the integration process of the implant in the body. The surface charge of a biomaterial is among others determined by the pH and the composition of the surrounding fluid, the chemical composition of the biomaterial surface, and the inflammatory situation in the body. Therefore, it is essential to gain a better understanding of the influence of surface charge and surface chemistry on the adsorption behaviour of proteins in order to improve the biocompatibility and long-term functionality of implants.

The objective of this thesis is to investigate the influence of surface charge on the adsorption of proteins on commercially pure titanium (CP Ti), a frequently used biomaterial in orthopaedic and cardiovascular devices. Titanium is covered with a native oxide layer of a few nanometers. The presence of this oxide layer contributes to the high biocompatibility and the high corrosion resistance of titanium implants. The adsorption behaviour of human plasma fibrinogen (HPF) and hen egg white lysozyme (HEWL) on CP Ti is investigated with both *ex situ* and *in situ* surface analysis techniques, and *in situ* sensing techniques.

As a first step in our investigation, the conformations of HPF and HEWL adsorbed on CP Ti, covered with its native oxide, are studied for the first time on a molecular level with *ex situ* and *in situ* atomic force microscopy (AFM). In order to obtain high resolution images of the adsorbed proteins on CP Ti a chemical-mechanical polishing procedure is developed for CP Ti. Because most literature studies have been performed on model substrates, the experimental procedure for AFM used in this work is validated by using mica as a reference material. When the conformations of HPF and HEWL adsorbed on CP Ti are compared with those on mica, a different adsorption behaviour is observed between HPF and HEWL for each substrate, and between CP Ti and mica for each protein. These observations show that the adsorption of HPF and HEWL is strongly determined by the surface properties of the substrate, i.e. the isoelectric point and the hydrophilicity. Based on the obtained AFM images on CP Ti and mica, adsorption models are put forward for HPF and HEWL on CP Ti.

The next step is the investigation of the influence of surface charge on the adsorption behaviour of HPF and HEWL on CP Ti. The approach used in this thesis is to keep the environmental conditions constant and to apply an external potential to the CP Ti substrate. In order to achieve this goal, *in situ* surface sensing techniques combined with electrochemical methods are developed and/or optimised for the real-time detection of protein adsorption on CP Ti in a range of applied potentials around open circuit potential (OCP). Because Ti is employed as a substrate, its oxidation behaviour needs to be taken into account. Ti is always

covered with a native oxide layer that can grow when a potential is applied. Therefore, in order to distinguish the adsorption phenomena of proteins and the oxide growth under applied potential, a preparation procedure of Ti samples with a stable oxide layer is successfully established.

The adsorption experiments of HPF and HEWL on CP Ti under the influence of an applied potential are performed by means of *in situ* ellipsometry combined with electrochemical measurements. One part of these experiments is done at OCP on CP Ti covered with its native oxide, the other part is performed at 0 V, OCP and 0.6 V on CP Ti covered with its stable electrochemically grown oxide. The latter potential range is considered representative for possible potential variations around OCP of the CP Ti implant in the body environment. It is observed that the adsorbed mass of both HEWL and HPF on the native and electrochemically grown oxide are strongly dependent on the protein concentration. After electrochemical growth of the titanium oxide layer, the range of applied potentials has no significant effect on the adsorption of HPF and HEWL for the investigated protein concentrations. We think that the main reason for this behaviour is the lower electronic conductivity of the oxide layer after electrochemical oxide growth. In the range of applied potentials, the variation of the surface charge was insufficient to influence the electrostatic adsorption of HPF and HEWL. Based on the *in situ* ellipsometry results, adsorption models related to the theoretical monolayer coverage model are put forward for HPF and HEWL adsorbed on CP Ti. The models show that water is coupled to the adsorbed films coming from direct hydration and trapped water in between molecules. The presence of coupled water and its influence on the adsorption behaviour of HPF and HEWL at an applied potential near OCP is investigated by means of electrochemical quartz crystal microbalance with dissipation (ECQCM-D). With this technique, the amount of coupled water and the viscoelastic behaviour of the adsorbed protein films are determined. Because of experimental limitations of the ECQCM-D setup, Ti coated QCM crystals are prepared and employed instead of the CP Ti samples. It is observed that the adsorption behaviour of HPF and HEWL is strongly influenced by the size and the native conformations of the adsorbed proteins and the amount of coupled water. It is also observed that the rigidity of the adsorbed HPF and HEWL films is not changed after electrochemical growth of the oxide nor by applying the studied range of potentials to the substrate.

Based on the complementary information obtained with *in situ* ellipsometry and ECQCMD, complete adsorption models are put forward for HPF and HEWL on the native and electrochemically grown titanium oxide.