

HEMOCOMPATIBILITY OF HYDROXYAPATITE

INTRODUCTION

Hydroxyapatite (HA) coatings are generally considered to improve the osseointegration of biomedical implants. In this example a nanocrystalline HA surface was compared to Titanium, a common implant material, and Chromium as negative control. It is generally assumed that selective adsorption of plasma proteins to the implant surface material is a critical factor for plasmonic and cellular activation pathways. Several of these proteins are present in blood plasma (von Willebrand factor, fibrinogen, fibronectin, for instance). Even after a very short exposure time, these proteins bind to the artificial surfaces which will influence further interactions with integrins and activation pathways. It has previously been shown by Weber et al (J. Biomed. Mater. Res. 2005) that a model system of fibrinogen and the major platelet membrane receptor (GPIIb-IIIa) can be used to predict the performance of the hemocompatibility of polymeric biomaterials. This approach was repeated here with the novel HA surface and a reference TiO_x surface.

EXPERIMENTAL

Here we present a novel approach for the formation of the HA coating onto sensor crystals using the Promimic HA^{NANO} Method, developed and patented by Promimic AB, Sweden. We also present unique real time measurement data where these coating have been used as a substrate for use with the Quartz Crystal Microbalance with Dissipation (QCM-D) technique. The HA surface was

characterized with X-ray Photoelectron Spectroscopy (XPS), Atomic Force Microscopy (AFM) and Quartz Crystal Microbalance with Dissipation (QCM-D). The thickness of the HA layer was found to be approximately 10 nm and uniformly distributed over the surface. The coating was stable and showed practically no drift during the QCM-D measurement (figure 1), a prerequisite for reproducible QCM-D studies. The HA surface was

compared with TiO_x, a commonly used implant material, and Chromium (negative control). The surfaces were cleaned by UV/Ozone treatment, rinsed with milliQ water, blown dry with Nitrogen gas and inserted into the measurement chamber. After establishing a stable baseline in buffer the surface was exposed to first fibrinogen, a blocking step of high concentration of Albumin, followed by the platelet receptor GPIIb-IIIa.

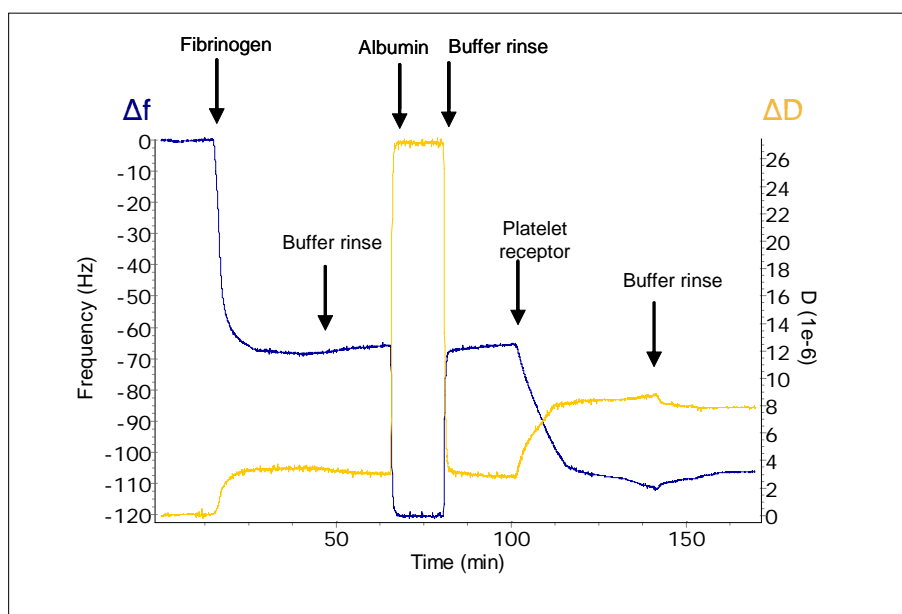


Figure 1 – Protein adsorption to HA. TRIS buffer (50 mM TRIS, pH 7.4 at 37°C containing 100 mM NaCl, 1 mM MgCl₂, 1 mM CaCl₂). Fibrinogen concentration 50µg/ml, BSA 10%w/v, GPIIb-IIIa 10 µg/ml

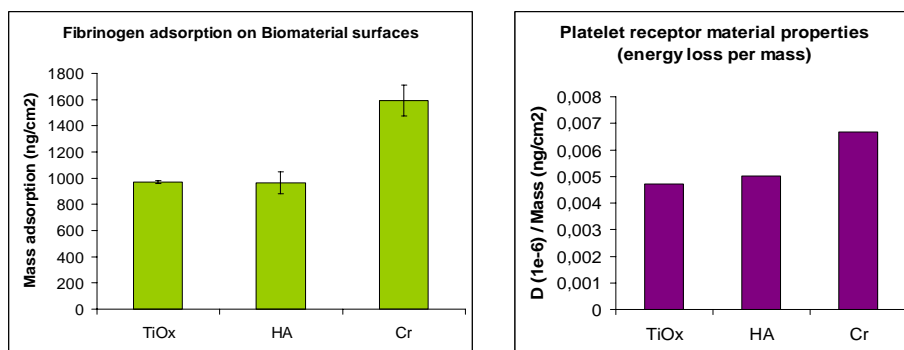


Figure 2 – Results from the protein and platelet adsorption studies. Every experiment was repeated twice and the error bar represents 2 standard deviations

RESULTS

All the surfaces quickly reached stable baselines (figure 1) indicating that the surfaces were stable and did not dissolve. The HA and TiO_x surfaces showed similar behavior (figure 2) when exposed to fibrinogen, while the Cr surface showed a much higher adsorption. It is believed that the conformation of the fibrinogen is an important factor as well as the total amount, when it comes to determining the hemocompatibility of a biomaterial. After the blocking step of Albumin, the platelet receptor again binds differently to the Cr surface compared to the HA and TiO_x (figure 2). The $\Delta D/\Delta f$ ratio shows the viscoelasticity per mass of the adsorbed layer and gives insight into the conformation of the molecules. It is evident that the Cr surfaces is significantly different than the HA and TiO_x surfaces.

CONCLUSION

In this study we have shown the use of HA coated QCM-D crystals to study the hemocompatibility of HA compared to the known biomaterial TiO_x and the negative control Cr. The HA surface proved to be suitable for hemocompatibility studies and results are well in accordance with previous work.

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