Kinetics, conformation, and two-step competitive protein adsorption studies with TiO_2 crystals using QCM-D.

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Introduction: Quartz crystal microbalance with monitoring of dissipation (QCM-D) [1,2] was used in this study to obtain real time qualitative and quantitative information not only on the amount of adsorbed/desorbed proteins but also to investigate the conformational changes and/or the water uptake of the adlayer on TiO_2-coated crystals. Single protein studies of key proteins (fibronectin, fibrinogen, and albumin) for the biological performance of titanium implants in its different biomedical applications and two-step competitive studies of these proteins have been carried out. This leads to gain further fundamental insight into the complex behavior of protein adsorption on TiO_2 surfaces, i.e., in the first biological events when a titanium implant is placed in contact with living tissues.

Objective: Obtaining a comprehensive real-time description (kinetics, conformation, and competition) of key-protein/TiO_2 interactions using QCM-D technique.

Materials and Methods: A QCM-D Q-Sense D300 System (Sweden) was used for all the protein-interactions tests. Sensors of quartz coated with TiO_2 with a fundamental mode of 5 MHz were used. The crystals were cleaned with Hellmanex (2%) (20'), ethanol (10') and distilled water sonication (10'). Finally, sensors were placed 10' into a UV/Ozone chamber. Changes in both resonance frequency (Δf) and energy dissipation (ΔD) of TiO_2-coated sensors along the time (t) were determined by exposition for 4 hours at 37 ^oC to 40, 80 and 100µg/ml of Fn-, Fbn- and BSA-solutions, respectively. Adsorbing the first-protein, washing into the microbalance chamber twice with PBS 1X, and proceeding with the second-protein was the protocol followed for two-step competitive adsorption studies. The protein-concentration ratios reproduced the protein ratios of the human blood plasma.

Surface characterization of the TiO_2-coated sensors by means of SEM and contact angle (CA) measurements with ultrapure-MilliQ water was performed.

Results and Discussion: SEM images showed a homogenously smooth TiO_2 surface of the crystals. Wettability studies indicated a CA of 55.0º±4.4 for TiO_2 crystals. After the cleaning procedures, there were not statistically significant differences in the CA of the surfaces.

Δf–t and ΔD-t plots obtained with different concentrations of mono-protein solutions, provided information about how mass (Δm) and viscoelastic properties of the adlayer changed and were related during adsorption. The increasing concentration increased the mass adhered to the crystal and consequently reduced the resonant frequency. The time needed to get a steady frequency, i.e., when no more proteins attach to the surface, depended not only on the protein molecular weight but also on the protein spreading/conformation on the surface. Fn and Fng reached this point significantly earlier than BSA.

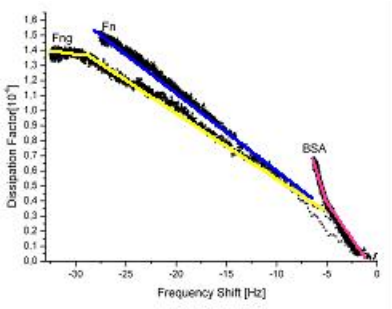

Fig. 1. ΔD-Δf plot of Fn, Fng and BSA adsorption on TiO_2 crystals.

The ΔD-Δf plot estimates how the new mass added affects the structure of the surface. For all the studied concentrations, Fn presented a single phase during time (Fig.1). The changes in the slope of these curves during Fng- and BSA-adsorption indicated changes in the rigidity of the adlayer. These changes demonstrate a time dependency of the conformation and/or water uptake of the proteins adsorbed.

The ratio ΔD/Δm was calculated to evaluate the amount of water trapped in the protein film [3]. For all the proteins studied the initial ΔD/Δm value was the same but BSA showed an increase of this ratio value with time, which indicates an increased amount of water captured. Fn showed a constant relation and Fng a slightly decrease with time.

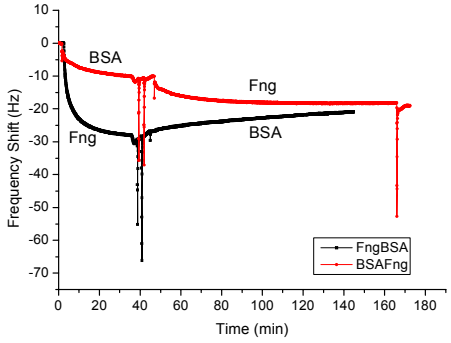

***Fig. 2**. Δf-t plot of two-step Fng- and Alb-adsorption.*

Competitive two-step protein adsorption studies showed that both, BSA and Fn pre-coating significantly reduced and decelerated Fn and Alb adsorption, respectively, compared to the amount and speed of adsorption in single protein studies. Fng was displaced by BSA showing a higher affinity of BSA for being adsorbed on TiO_2-surfaces (Fig. 2).

Conclusions: Fn and Fng adsorb faster on TiO_2-surfaces than BSA, but when in competition, BSA displaces Fng. The monoprotein Fng and BSA adlayers suffer changes in its conformation and/or water uptake with time.

References: [1] Andersson, M. et al. (2005) *Biosens. Bioelctr.* 21, 79-86. [2] Hemmersam, A. et al (2005) *Coll. Surf. B* 43, 208-15. [3] Höök F. et al.(2002), *Coll.Surf. B* 24, 155-170.