Cascade Reactions of Blood at Model Biomaterial Surfaces

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ABSTRACT

A new and simplified methodology for quantification of Immune Complement (IC) and contact associated Blood Coagulation (BC) activation potential biomaterial surfaces is described in this thesis. The method is based on that activation of the IC and BC systems are made at the sensor surfaces of Quartz Crystal Microbalance with Dissipation monitoring (QCM-D), an acoustic method sensitive to adsorption of proteins to solid surfaces.

In **paper I** we showed that IC activation could be conveniently quantified by measuring the amounts of deposited anti-C3c at the sensor surface, previously incubated with serum. We also show that the sensitivity for the detection of adsorbed complement protein with the QCM-D was in the same range as Surface Plasmon Resonance (SPR), a surface sensitive optical method.
In **paper II** we quantified fibrin deposition at the QCM-D sensor surface by placing citrated plasma on the sensor surface followed by addition of calcium (Ca^{2+}). After a lag time, fibrin formation at the surface was detected by a shift in frequency and visible detection of formed fibrin clots. Titanium, which is a highly thrombogenic surface, had the highest rate of fibrin formation. Heparin functionally surfaces, which is an actively anti thrombogenic surface, displayed no fibrin clot formation during plasma incubation.
In **paper III** we studied the effect of substrate molecular mobility on surface induced IC and BC activation. A series of spin-coated poly(alkyl methacrylate) polymers with different glass transition temperatures (T_g) were analysed. Although the molecular flexibility varied, the surface energy was about the same on all the surfaces. It was found that increased molecular flexibility correlated with a decrease in IC activation but no correlation between molecular flexibility and fibrin clot formation, BC activation, was found.
In **paper IV** we made a more detailed investigation of the relation between QCM-D measurements of C3-fragment deposition and determination of C3a and soluble Terminal Complement Complex (TCC). We found that the affinity, structure and viscoelasticity of adsorbed layers of IgG are of importance for IC activation.
In **paper V** we made a more systematic study of the similarities and the dissimilarities between surface sensitive methods as QCM-D and enzyme immuno assay (EIA), which is based on measurements of soluble products. Methods like EIA and cell counting is based on quantification of soluble factors in the IC and BC systems. We used titanium, heparin functionally surfaces and polymers commonly used in medical implants. We also studied the structure of the fibrin clot formed on the model surfaces using D/f-plots.

Keywords: QCM-D, IgG, protein adsorption, immune complement activation, blood plasma coagulation, polymers, SPR, spin-coating, glass transition temperature

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