

Analyzing surface induced complement activation

This application note shows how QCM-D can be used to study immunogenicity of biomaterials. Biomaterials introduced into the body are known to cause surface associated complement activation in human sera, which triggers inflammation. By exposing different surfaces to human serum containing complement proteins, aspects of blood compatibility of the materials can be evaluated. Here, the blood compatibility of different biomaterials are quantified as amount of bound C3 convertase.

Quartz Crystal Microbalance with Dissipation (QCM-D) is an acoustic surface sensitive technique, which provides simultaneous, real-time information on mass and structure of molecular layers. With the QCM-D technique it is possible to make accurate determinations of protein interactions on different biomaterial surfaces in real-time. Here we show how QCM-D can be a useful tool in evaluating the immunogenicity of different biomaterials.

Introduction

When a biomaterial is introduced into the body, protein adsorption and activation of complement proteins occur. Complement proteins are mediators of inflammation, and are therefore of interest in biomaterials research. Part of the surface induced activation of the complement system is C3 convertase, which adsorbs to surfaces and can be detected using QCM-D.

Approach

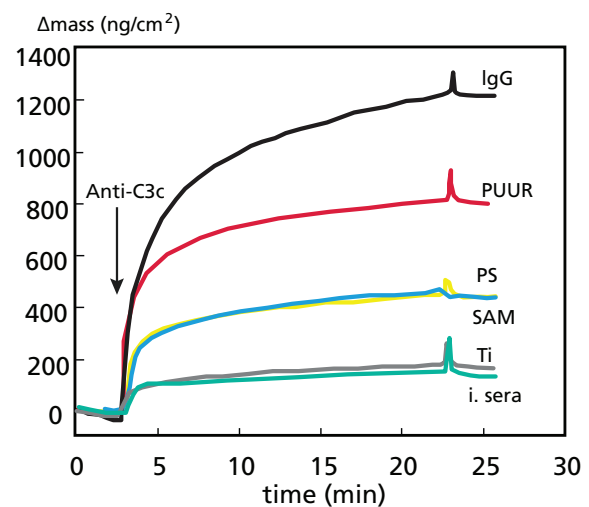
The following surfaces were prepared for the investigation:

1. IgG, positive control
2. Polyurethane urea (PUUR)
3. Polystyrene (PS)
4. Hydrophobic self-assembled monolayer (SAM)
5. Titanium dioxide (TiO₂)
6. Heat-inactivated sera, negative control

The surfaces were prepared on Q-Sense gold sensors. Prior to the measurement, surfaces were incubated with human serum containing complement proteins. The pre-incubated sensors were then exposed to rabbit anti-C3 convertase antibodies in situ to quantify the amount of adsorbed C3 convertase and to study the antibody binding in real-time.

Results & Discussion

Figure 1 shows the detected amount of anti-C3 convertase antibodies on the different surfaces. Binding was observed on all surfaces, though in differing amounts. As expected, complement activation was found on the pre-adsorbed IgG surface, which acted as positive control. Positive responses were also detected on PS and PUUR coated sensors. However, the degree of anti-C3 convertase binding on the TiO₂ surface was as low as on the negative control (heat-inactivated sera), indicating low immunogenicity of TiO₂. The order of complement activation (as judged from three separate measurements per surface modification) was PUUR>PS= SAM>TiO₂.



[Figure 1]: Binding of Anti-C3 convertase on different substrates, where sera is negative control, IgG is positive control.

Conclusions

This work shows that QCM-D is a suitable method to study blood compatibility of different biomaterial surfaces. Real-time measurements of the amount of adsorbed proteins is a unique approach to screening biomaterial immunogenicity. The flexibility of QCM-D in allowing use of different surface materials (metals, polymers or chemically modified surfaces) without need to calibrate and without affecting the sensitivity, is an important benefit in the field of biomaterial research.

References:

Sellborn, A, Andersson, M, Fant, C, Gretzer, C and Elwing, H (2003) Methods for research on immune complement activation on modified sensor surfaces, *Colloids and Surfaces B: Biointerfaces* 27, 295-301.



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