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Quartz crystal microbalance-with dissipation monitoring (QCM-D) for real time measurements of blood coagulation density and immune complement activation on artificial surfaces

Marcus Andersson[a,*], Jonas Andersson[b], Anders Sellborn[a], Mattias Berglin[a], Bo Nilsson[b], Hans Elwing[a]

[a] *Department of Cell and Molecular Biology/Interface Biophysics Lundberg Laboratory, Göteborg University, Box 462, SE-405 30 Göteborg, Sweden*
[b] *Department of Oncology, Radiology and Clinical Immunology, Section of Clinical Immunology, Rudbeck Laboratory C5, Uppsala University Hospital, SE-751 85 Uppsala, Sweden*

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Abstract

A recently developed variant of quartz crystal microbalance (QCM) called QCM-with dissipation monitoring (QCM-D) allows simultaneous and simple measurements of changes in adsorbed mass as well as the viscoelastic property (D-factor) of deposited protein layers on the sensor surface. We have taken the QCM-D technology a step further and demonstrated its advantages in the study of protein assembly as a consequence of surface induced immune complement activation, or contact activated blood coagulation. In the present study we have continued our QCM-D investigations of surface assembly of fibrin clot formation and complement activation and incubated differently modified quartz sensor surfaces in blood plasma and sera. Polymer surfaces used were spin-coated polyethylene, poly(ethylene terephtalate), poly(methylmetacrylate) and poly(dimethylsiloxane). Also used were sputtered titanium and heparin grafted surfaces. In this investigation we found that we could describe the surface induced coagulation with four independent parameters: (1) Time of onset of coagulation, (2) fibrin deposition rate, (3) total frequency shift at stable plateau, and (4) fibrin clot density. The most important finding was that the blood plasma clot density can be assessed with the use of *D* determinations and that the clot density varied significantly with the chemical composition of the surface. However, the D-factor did not give any new analytical information about the possible complement activation mechanisms. Nevertheless, the QCM-D was found to be a reliable tool for the analysis of surface induced complement activation.

We also compared the QCM-D technique with traditional enzyme immuno assay (EIA) measurements of soluble products from the surface activation of the complement and coagulation systems. We found that the results from EIA and QCM-D measurements corresponded well for the complement activation but not for the coagulation, probably due to the biological complexity of the coagulation system.

Keywords: Quartz crystal microbalance; Blood coagulation; Complement activation; Enzyme immuno assay

1. Introduction

Quartz crystal microbalance (QCM) is a high frequency surface sensitive method for various biosensor applications (Marx, 2003). Most applications monitor only changes of resonant frequency (Δf) of the adsorbed layers on the sensor surfaces. Recently, a variation of QCM has been developed which simultaneously allows registration of dissipation (D) of the sensor signal (Rodahl et al., 1995; Hook et al., 1998). The D-factor is related to the viscoelastic properties of the adsorbed layer. The new QCM modification, called QCM with dissipation monitoring or QCM-D, has greatly increased the analytical range of the instrument in different applications, for example in studies of single protein adsorption (Hook et al., 1998) and adsorption of phospholipid layers (Glasmastar

* Corresponding author. Tel.: +46 31 7732566; fax: +46 31 7732599.
E-mail address: marcus.andersson@gmm.gu.se (M. Andersson).

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et al., 2002). The benefit of simultaneous registration of *D* and *f* has been demonstrated in several investigations, where QCM-D measurements were combined with optical surface sensitive methods such as ellipsometry and surface plasmon resonance (SPR) (Hook et al., 2001).

Our present interest in QCM-D is mainly focused on measurements of protein adsorption to biomaterials. Previously, our group has used QCM-D to study complex protein–surface interactions such as immune complement activation and contact activated blood coagulation (Sellborn et al., 2003; Andersson et al., 2002; Berglin et al., 2004). Surface induced activation of the complement system and coagulation system result in a massive deposition of proteins on the surface, which is of great importance for biomaterial compatibility (Tang et al., 1998; Kalltorp et al., 1999). Traditional methods investigating complement and blood coagulation systems are mostly based on measurements of soluble products. The introduction of the QCM-D method presents a way of studying new mechanisms of bio-interface research.

Aim of this investigation was to refine the QCM-D technique for quantification of both the complement and the coagulation system on solid surfaces. Preliminary studies indicated that the clot density is dependent on the chemistry of the surface. Thus, our second aim was to perform a more detailed analysis of the D-parameter in relation to fibrin deposition. The QCM-D was used to investigate materials commonly used in medical implants such as titanium (Ti), polyethylene (PE), poly(methyl methacrylate) (PMMA), poly(ethylene terephtalate) (PET), poly(dimethylsiloxane) (PDMS) and heparinized surfaces (Hep). The QCM-D results were compared to results obtained from traditional methods such as enzyme immuno assays (EIA) of soluble factors and platelet counting.

1.1. Short description of the coagulation and complement systems in relation to biomaterials

Activation of the blood coagulation system on biomaterial surfaces may result in fibrin clots that may impair the function of various medical devices such as artificial blood vessels, stents (Christensen et al., 2001), blood catheters and other extra corporal devices. Blood coagulation kinetics and clot characterisation are studied in various ways such as spectrophotometry (Sanchez et al., 2002), QCM (Andersson et al., 2002), ellipsometry (Walivaara et al., 1996), SPR (Hansson et al., 2002; Vikinge et al., 2000a, 2000b) and free oscillation rheometry (Hansson et al., 2002). Platelets are important mediators in the coagulation cascade and are involved in surface induced blood coagulation. The platelet number is usually determined by cell-counting methods, and their degree of activity is measured by the amount of thrombospondin (TSP) released, using EIA or equivalent methods (Bergseth et al., 2000). In our setup, EIA is performed on blood that has been incubated on coated glass slides using the slide chamber model (Hong et al., 1999b, 2001). Measuring potential surface induced blood coagulation often involves measurement of the soluble thrombin–antithrombin complex (TAT) with EIA as the standard method. However, the QCM-D instrument has turned out to be a very interesting alternative for determination of surface induced blood coagulation, since the fibrin formation can be detected in situ and in real time on the inductive surface. The frequency shift gives information about the general clotting kinetics, such as time of onset and fibrin deposition rate, while the dissipation factor provides valuable data concerning viscoelastic properties, such as clot density. This way of measuring surface induced blood coagulation has been further refined in this study.

The immune complement system is present in blood and serum and consists of about 20 different proteins. On contact with a foreign surface, e.g. a bacterial surface, the complement system is activated in a cascade fashion, resulting in destruction of the bacterial surface or release of bioactive degradation products, causing inflammatory reactions in the surrounding tissue, or both. However, the activated complement cascade can be a major problem concerning medical implants and extra corporal blood treatment devices such as oxygenators, dialysators, etc. (Tang et al., 1998; Kalltorp et al., 1999). It also seems to have long term implications, e.g. for in wear debris from total joint arthroplasty (DeHeer et al., 2001) and xenotransplantation (Bengtsson et al., 1998). Determination of complement activity usually involves EIA measurements of fragments from degradation of complement factors in blood or serum that has been in contact with an activating surface. Examples of such products are complement factor 3a and 5a (C3a and C5a) and the terminal complement complex (TCC). Complement activation on a solid surface can also be detected by the amount of surface bound complement factor 3 (C3) on the activating surface. Surface associated C3 can be measured via anti-C3 antibodies, using a method such as ellipsometry (Elwing et al., 1986), surface plasmon resonance (SPR) or QCM (Sellborn et al., 2003).

2. Materials and methods

2.1. Surfaces

The following surfaces were used: titanium (sputtered on gold sensor surfaces in vacuum), polyethylene (Aldrich, WI, USA, 9002-88-4), poly(ethylene terephtalate) (Aldrich, 29154-49-2) poly(methyl methacrylate) (Aldrich, 9011-14-7), and poly(dimethylsiloxane) (Rhodia silicones, France, CAF 2534). Solvents and polymer concentrations are listed in Table 1. The polymers were spin-coated (spin-coater KW-4A Chemat Technology Inc., Northridge, CA, USA) on gold sensor surfaces (Q-sense, Göteborg, Sweden) using 50 μl polymer solution (polyethylene was heated to approximately 200 °C before casting). The thickness was estimated using QCM-D in air (Table 1). Titanium plates and microscope purpose glass slides coated with the above-mentioned polymers were used in the slide chamber model (see below). Surfaces