Electrochemical Techniques to Investigate Adsorption and Desorption Behavior of Fibrinogen on a Gold Surface

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Statement of Purpose: Electrode fouling or electrode encapsulation caused by protein adsorption is one of the major issues faced by metal-based biosensors in vivo. This study utilized an electrochemical quartz crystal microbalance (eQCM), scanning electrochemical microscope (SECM), and atomic force microscope (AFM) to evaluate the use of cathodic voltage-controlled electrical stimulation (CVCES) to promote fibrinogen desorption from gold substrates.

Methods: Electrochemical experiments were performed using potentiostat (Gamry Instruments, Reference 600) interfaced to a computer to monitor current-voltage signals. An eQCM (Gamry Instruments, eQCM 10M) flow cell with three electrode setup was used for monitoring mass change caused by adsorption and desorption of fibrinogen. The setup consisted of gold coated quartz crystal (working electrode), a platinum counter electrode, and an Ag/AgCl reference electrode. 0.9% saline at pH 7.4 was flowed through the eQCM flow cell system until a steady frequency shift was observed referred to as the baseline. Fibrinogen solution of 0.5 mg/ml concentration stored in a 1 ml aliquot was flowed through the system by opening the three-way valve while blocking saline flow. The fibrinogen adsorption was monitored by means of measuring the shift in resonant frequency of the oscillating quartz crystal sensor.

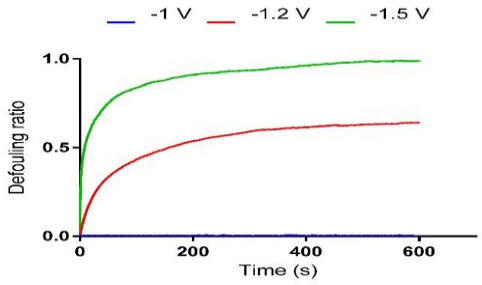

Fig. 1 Average defouling ratio of fibrinogen adsorbed on gold coated quartz crystal surface for applied CVCES of -1 V, -1.2 V, and -1.5 V (vs. Ag/AgCl) for 10 minutes.

Once the adsorption of fibrinogen reached saturation, the flow was reverted back to saline for rinsing. After the rinsing step, CVCES of -1 V, -1.2 V and -1.5 V (vs. Ag/AgCl) was applied for 10 minutes to the surface of gold coated quartz crystal. The frequency shift which corresponds to mass change caused by adsorption and desorption was monitored in real time using Gamry Resonator software. Defouling ratio was calculated for all voltage conditions to measure the relative amount of fibrinogen removed from total initial mass adsorbed on gold coated quartz crystal. SECM (CH Instruments 920C) was used for the hydrogen evolution and pH studies of the microenvironment close to the gold. A custom polycarbonate electrochemical cell that allowed for the positioning of the microelectrode sensors was used for the SECM studies. An amperometric detection method was used to qualitatively measure the relative amount of hydrogen being produced on the gold electrode as a function of voltage. Imaging of gold samples before and after CVCES was done in AFM. All the images were acquired in tapping mode. Parameters such as root mean square (RMS) roughness and surface coverage were analyzed using Asylum Research Software version 16.0 and ImageJ.

Results: Application of CVCES of -1.5 V to the gold surface resulted in 97.9% desorption of the initially adsorbed protein, while -1.2 V caused 63.2% desorption, and -1 V produced only 5.6% desorption. Potentiodynamic study of gold pointed to hydrogen evolution at voltage more cathodic than -1.2 V. Substantial removal of fibrinogen at voltage more cathodic than -1.2 V, demonstrated by eQCM is shown by their defouling ratio in Fig. 1. Acquired AFM images of gold QCM sample after analysis showed that surface coverage of fibrinogen after application of CVCES reduced from ~10.8% to ~5.2% (for -1.2 V) and ~0.2% (for -1.5 V) respectively. Amperometric hydrogen evolution study revealed a significant decrease in hydrogen sensor current in the presence of fibrinogen indicating hydrogen involvement in removal of fibrinogen at the cathodic potentials mentioned above. However, this study doesn't involve monitoring the structural change in fibrinogen. Hence, the understanding of hydrogenated and fragmented fibrinogen byproducts from CVCES are very limited. Also, only fibrinogen and its response to CVCES was tested for here. However, blood plasma has many other factors and glycoproteins responsible for thrombosis are not accounted for here.

Conclusions: The eQCM studies showed that CVCES promoted desorption of fibrinogen from gold substrates in a voltage dependent manner. The SECM studies determined that the water reduction reaction, which generates hydrogen along with pH change, plays a key role in the removal of fibrinogen. Topographical images from AFM, confirmed the removal of fibrinogen with application of CVCES.