

Parameters of hemostasis measurements like "Quick Time" (prothrombin time), have primary significance in many clinical settings including extensive surgery, dialysis or innate disorders of hemostasis. Real time measurements of prothrombin time by qCell T has been demonstrated to be an alternative method to conventional coagulometer.

Summary

Recently, several reports have documented the principle suitability of Quartz Crystal Microbalance (QCM) for measuring parameters of hemostasis like prothrombin time (PT) or platelet aggregation. But for the establishment of an exact QCM based method as an alternative to a standard coagulometer measurement, QCM coatings with significantly enhanced robustness and reusability have to be worked out. This application note demonstrates that affinity based polyethylene nano-particles (NP) absorbed to polymer film on the QCM constitute a powerful tool with no need for pretreatment for measuring PT in whole blood samples in real time, while these coatings are reusable [1]. PT in the range of standard coagulometer tests pave the way for possible future application of QCM in clinical routine.

Background

Hemostasis monitoring during surgical operations has fundamental significance especially in cardiac surgery involving extracorporeal circulation devices (Fig. 1). In such procedures blood parameters can drastically change within the lapse of a few seconds to minutes due to exposure of patients' haemostatic system to large disturbances such as haemo-dilution or anti-coagulation. Additionally some unwanted interactions of the blood with artificial surfaces may cause coagulation activation, blood platelet aggregation [2] or proinflammatory effects [3,4]. In such conditions frequent monitoring of the patients' hemostasis status is often crucial for suitable therapeutic directions and decisions [5,6].

Strategy

By measuring resonance frequency as well as dissipation, the instrument itself has a wide range of applications. Mass changes and properties as density and viscosity of attached layers and wetting liquids can properly be measured under well controlled conditions in liquid phase. The adaptation for measuring coagulation parameters is achieved by adequate coating of the QCM. This application note demonstrates the use of affinity-based NP adsorbed to polymer thin film for PT measured by the QCM method. As reference method for experiments mechanical coagulometry has been employed, which can presently still be considered as gold standard. This study thus mainly focuses

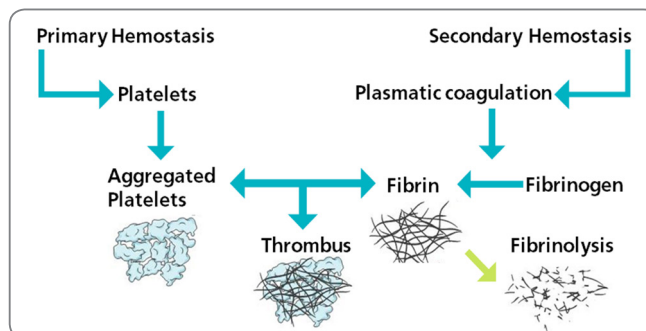


Figure 1. Blood coagulation. When a human vessel is damaged, platelets aggregate at the wound and form a plug to cover the vessel wall from bleeding (Primary Hemostasis). Simultaneously, Fibrin strands converted from Fibrinogen strengthen the plug (Secondary Hemostasis).

on the QCM technique as alternative to that of standard mechanical coagulometry. QCM technique differs from standard coagulometry in the way that it monitors the whole process of coagulation instead of measuring the end point of PT, only.

Method

QCM sensors were cleaned first with acetone followed by deionized water and dried under a stream of nitrogen. For further subsequent cleaning, sensors were treated with piranha solution for 1 minute, rinsed with deionized water and dried in a stream of nitrogen. After cleaning the sensors, the sensor electrode was spin coated at 2500 rpm for 20 seconds with 5 μ l mixture of 1-vinyl-2-pyrrolidone - di vinyl benzene (VP-DVB) (1:2 w/w). Subsequently, PE-NP suspensions in 7 μ l ethanol were added on unhardened thin film of monomer: cross-linker (1:2 w/w) and spin coated at 2500 rpm for 40 seconds. The spin coated sensors were incubated at 38 $^{\circ}$ C for 12 hours for immobilization and hardening the NP on polymer prior to real time PT measurements on qCell T. QCM containing NP on polymer layer was inserted into the qCell T and rinsed with Tris buffer (pH 7.4) at 37 $^{\circ}$ C prior to real time PT measurements. The citrated whole blood mixed with heparin (0, 1.0, 1.5, 2.0 IU/ml) to achieve different PT ranging from few seconds to tens of seconds have been used. 100 μ l of the according blood sample was incubated at 37 $^{\circ}$ C for 1 minute in the incubation chamber of the qCell T. Then, 200 μ l thromborel, freshly prepared and incubated at 37 $^{\circ}$ C, was mixed with whole blood sample. The mixture was

immediately pumped to the measuring chamber at a flow rate of 0.8 ml/min via running the script control option. The PT with the mechanical coagulometer were performed with equal mixing ratios, volumes and incubation times.

AFM images of NP were measured with a VEECO Instruments Nanoscope IVa using contact mode with silicon nitride tip (Fig. 2 A). NP in absolute ethanol were spin coated at 2500 rpm on glass slides. The spin coated NP glass slides were incubated at 38 °C for 12 hours for drying and immobilization prior to AFM measurements. PE-NP surface morphology depicts that individual NP have an average diameter of around 150–200 nm. After NP characterization by using AFM, QCM sensors were applied for PT measurements by using the sensor platform as mentioned above. Measurements with and without induced coagulation can clearly be distinguished from one another with respect to frequency and dissipation shifts and shapes of curve (Fig. 2 B).

The qCell T is a thermostated instrument that can monitor every step including dissipation spectrum (half of the half bandwidth of the Lorentz-Curve) in addition to the frequency response (both in Hz). In order to compare the results obtained from the QCM measurements with those of a standard coagulometer, the end point of the PT has to be determined by picking a significant point of the frequency curve. This point is the local minimum where stabilization of the frequency after abrupt shift as depicted in Fig. 2 is achieved and defined it as t_{QCM} .

In Fig. 3, the data explains the values for citrated whole blood and blood with the addition of Heparin (0, 1.0, 1.5, 2.0 IU/ml), respectively. Ideal correlation between the two methods is represented by the line through origin. The results are found to

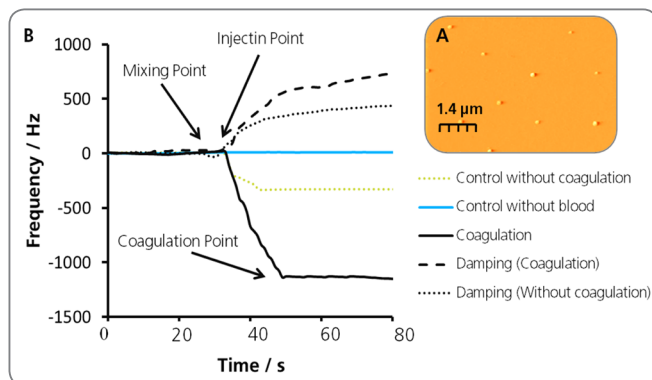


Figure 2. A. AFM image of PE-NP in ethanol. NP diameter 150–200 nm. B. Exemplary measurements of whole blood PT including damping curve as well as two different negative controls: whole blood without blood coagulation and coagulation activator without blood for NPs immobilized on VP-DVB.

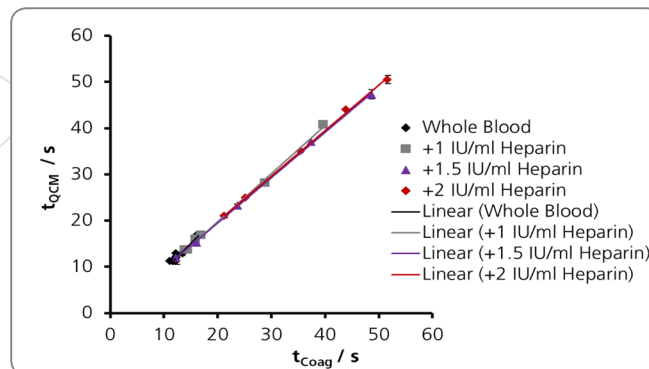


Figure 3. PT of human whole blood obtained from the QCM system plotted against PT measured with a mechanical coagulometer. Whole blood samples and whole blood samples with different heparin concentrations are indicated with appropriate symbols for NPs immobilized on VP-DVB. Each data point represents the mean of two measurements demonstrating error bars \pm SD of two measurements.

be within the specified tolerances. Fig. 3 depicts that the data located on the bisecting line represent an excellent correlation with the mechanical coagulometer.

Conclusion

PE-NP coupled with VP-DVB polymer system was monitored for recognition of PT of whole blood by the thermostated QCM instrument, qCell T. Comparative measurements of probes spiked with different concentrations of unfractionated heparin as anticoagulant revealed an excellent correlation of QCM based measurements to standard coagulometer measurements under analytical deviation limits. Utilizing qCell T has advantages over other conventional methods, especially in perspective of point of care application. Moreover the quartz sensor exhibited excellent reusability during experiments for three months, demonstrating no loss in characteristics.

Reference

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