Application Note
Platelet Aggregation Monitoring

Using a qCell T under stop flow conditions it was possible to measure blood platelet aggregation on fibrinogen coated sensors. The qCell T measurements were in good accordance with the established gold standard method of optical platelet aggregometry.

Summary

Monitoring of platelet aggregation with a mass sensitive qCell T device provided information of platelet aggregation and adhesion processes. It was possible to gain real time insight into the process of platelet aggregation and surface adhesion. The qCell T contains a mass sensitive quartz sensor mounted in a temperature controlled flow cell. The sensor responds with alterations of resonance frequency to changes in mass deposition to the sensor surface due to platelet aggregation and adhesion processes. The results were in good accordance with the established clinical method of platelet aggregometry. This study for the first time showed the proof of principle that platelet aggregation can be correctly measured by a QCM based test system.

Background

In cardiac surgery involving extracorporeal circulation and open heart surgery, haemostatic parameters can change within minutes. Therefore the attending physician has to make crucial decisions about the transfusion of blood products or altered anticoagulation therapies. This requires profound information of the patient's blood coagulation status. Beyond tests for plasmatic clotting time and fibrinolysis, profound information about platelet functionality is of major importance.

The challenge of our approach was to adapt a 3T analytik qCell T quartz crystal microbalance system for platelet aggregation measurements. Since the qCell T has proven its suitability for measurements of plasmatic coagulation in previous experiments [1], the desired benefit would be measuring different parameters with the same device.

Strategy

In a QCM system, fibrinogen-coated sensors or bare gold sensors were incubated with Adenosine diphosphate (ADP) or arachidonic acid (AA) stimulated platelet rich plasma. For negative controls the GPIIb/IIIa inhibitory antibody abciximab (Reopro) was used as an inhibitor of platelet aggregation. During incubation, the frequency shifts of the sensors were recorded. The results gained from the QCM system were compared to results gained by optical platelet aggregometry (born aggregometry). For additional visualization of platelet adhesion to the sensor surfaces, fluorescent microscopy and scanning electron microscopy were used. In this application note, only fibrinogen experiment is described. More details can be found in reference [2].

Method

Chemicals and blood sample preparation. Fresh human whole blood was collected and centrifuged for separating platelet rich
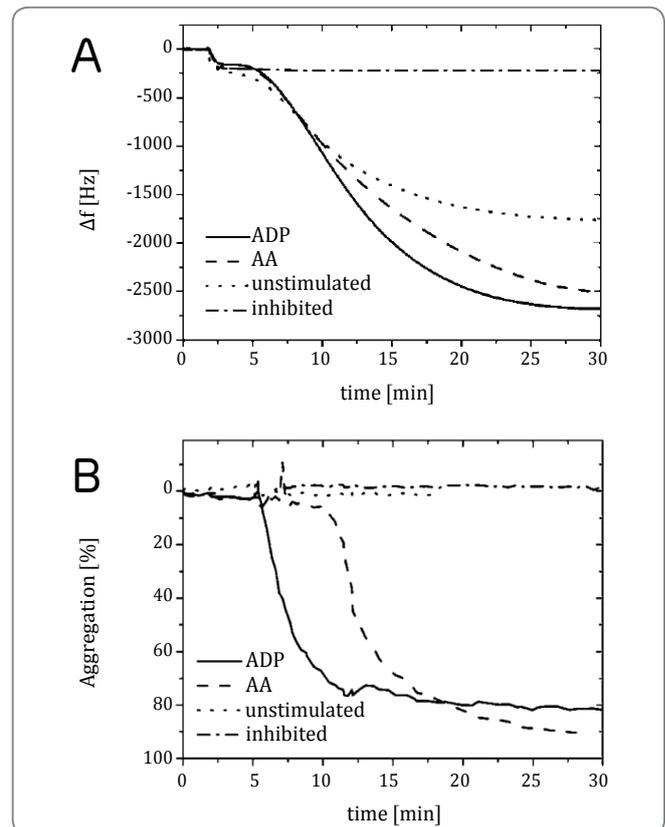

Figure 1. A). Platelet aggregation experiments was carried out with stimulation of ADP or AA on fibrinogen coated gold sensor. Negative control with GpIIb/IIa inhibitory antibody abciximab (Reopro). B). Results from conventional optical aggregometry.

plasma (PRP). After the PRP was carefully extracted, the remaining blood was centrifuged again for separating platelet poor plasma (PPP). PRP was adjusted at 300 000 PLT per ml by mixing platelet rich and platelet poor plasma.

Sensor and Platform. The sensor platform was kept at a constant of 37 °C for simulating in vivo conditions. Sensors were coated with fibrinogen. Fibrinogen coating allows the selective binding of platelets via GPIIb/IIIa receptors.

QCM sensors were first cleaned with acetone for 1 min, then rinsed with deionized water and dried under a stream of nitrogen. The sensors were cleaned for 1 min in Piranha solution and then rinsed with deionized water again. Next the sensors were again dried in a stream of nitrogen.

For fibrinogen coating, after the cleaning procedure, the sensor surfaces were treated with 11-mercaptoundecanoic acid solution which provides functional carboxy groups attached to the gold surface for the following protein coupling. Then, a mixture of

EDC/NHS solution was applied to the sensor surface generating a semistable NHS-ester. In the next reaction step, the NHS-esters react with amine groups of the added human fibrinogen, which leads to covalently bonded fibrinogen on the quartz surface. **Platelet aggregation.** ADP and AA were used as stimulants for platelet aggregation. Abciximab was used to inhibit platelet aggregation and adhesion via GPIIb/IIIa involvement.

Results and Discussion

The fibrinogen coated sensors responded to PRP contact with an initial drop of resonance frequency approximately 195 Hz. In the following 20 min a further frequency decreases took place. After 20 to 30 min the main part of the frequency decrease was over and subsequently only marginal decreases in resonance frequency took place (Fig. 1A).

For unstimulated controls, the initial frequency shift was about 240 Hz; after 30 min, the frequency values declined about 1800 Hz (Fig. 1A).

In negative controls for complete inhibition of GPIIb/IIIa binding sites, 40 µl/ml abciximab was used [3]. After the addition of abciximab to the PRP, the platelets were stimulated with either AA or ADP. Inhibition of GPIIb/IIIa leads to impaired aggregation and adhesion. Therefore, the decreases that can be observed in

resonance frequency are much lower than in solely ADP or AA treated positive controls. (Fig. 1A).

Aggregometer data were used as a reference for the QCM measurements. The aggregometer measurement curves are shown in Fig. 1B. Only in ADP and AA stimulated samples platelet aggregation was detectable. In samples with additional abciximab as inhibitor, as well as in unstimulated samples, nearly no platelet aggregation was detected.

In addition to scanning electron microscopic images, fluorescent microscopy was performed. In the ADP and AA stimulated samples nearly the whole sensor surface was covered by adherent platelets. FM images of ADP and AA treated fibrinogen sensors showed surface coverage with fluorescent platelet aggregates. According to the SEM images of the fibrinogen surfaces, ADP treated samples seemed to exhibit denser platelet aggregates, forming more compact clusters, whilst the AA treated samples seemed to form more widespread, less compact platelet aggregates.

The sensor surface in unstimulated samples is also covered with platelets. In comparison with ADP and AA treated samples, the platelet aggregates are less dense. The addition of abciximab almost completely inhibited the adhesion of platelets to the sensor surface. After stimulation with ADP or AA, only loosely scattered platelets could be found on the sensor surface.

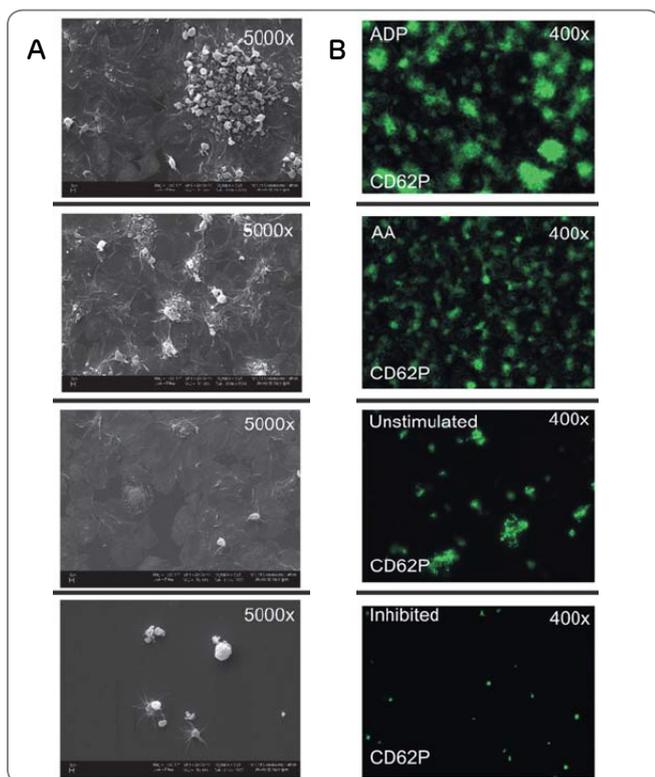**Figure 2.** Scanning electron microscopic images (A) and fluorescent microscopic images (B) of fibrinogen coated sensors.

Conclusion

This study for the first time showed the proof of principle that platelet aggregation can be correctly measured by a QCM based test system qCell T. The therapeutic diagnosis of platelet function with both qCell T and optical systems would achieve the same results for stimulated and inhibited samples. QCM based platelet aggregation monitoring has the potential to evolve into an alternative to current methods for platelet function testing.

Reference

[1] Müller L, et al, Investigation of prothrombin time in human whole blood samples with a quartz crystal biosensor. Anal. Chem. 82 [2], 658-663. 15-1-2010.

[2] S. Sinn, et al. Platelet aggregation monitoring with a newly developed quartz crystal microbalance system as an alternative to optical platelet aggregometry, Analyst, 2010, 135, 2930-2938.

[3] A. E. Asberg and V. Videm, Concentration-dependent effect of abciximab on platelets and neutrophils in a model of cardiopulmonary bypass, Perfusion, 2006 Nov, 21(6), 343–352.

Acknowledgement

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