The interaction of bacteria with platelets and plasma proteins is a critical step in the build-up of bacterial biofilms on for example prosthetic or damaged heart valves. A method based on QCM device-qCell T was successfully established to investigate the interaction in real time.

**Summary**

The interaction of bacteria with platelets plays a pivotal role in for example the pathogenesis of life-threatening infective endocarditis (IE) or prosthetic heart valve infections. We have successfully established a method to investigate the interaction of bacteria with platelets and plasma proteins on the basis of the quartz crystal microbalance (QCM) technique. Using the qCell T device, real-time investigation of platelet-bacteria interactions under a defined flow speed and temperature could be performed.

**Background**

The QCM is a highly sensitive technique based on the piezoelectric effect, which can be applied for real-time analysis of molecular mass adsorption. Furthermore, the frequency and damping signals can give information about the viscoelastic properties of the surface-bound layers.

The interaction of bacteria and platelets is a critical step in the build-up of bacterial biofilms on for example prosthetic or damaged heart valves resulting in complications like IE [1, 2]. The underlying molecular mechanism is, however, not completely understood.

**Strategy**

Once, a stable baseline curve is established, platelets are allowed to adhere to the surface of the quartz sensor under a constant flow speed. After a subsequent washing step to remove non-adherent platelets, a bacterial suspension can be perfused over the platelet layer and platelet-bacteria interactions can be investigated by means of damping and frequency shifts. After each measurement, the adhesion of platelets and bacteria can be confirmed using microscopic analyses.

**Method**

1. Platelet isolation: Blood from healthy donors was collected by venipuncture and platelet-rich plasma (PRP) was prepared by centrifugation of anticoagulated whole blood.

2. Preparation of bacterial suspension: Bacteria was grown in brain-heart infusion medium overnight at 5% CO₂ and 37°C. For the adhesion experiments, the bacteria were resuspended in PBS to get a final concentration of $1.0 \times 10^8$ CFU per ml.

3. QCM preparation: Before each experiment, gold-coated quartz crystals were cleaned with 70% ethanol. After mounting the sensor into the flow cell, the system needs to be primed with phosphate-buffered saline (PBS, 1M, pH 7.4) and stable frequency and damping signals are established at 22°C under flow in PBS requiring up to 15 min.

4. QCM measurement: After stable baselines are achieved, PRP is perfused over the sensor surface at 60 µl/min and 22 °C for 30 min followed by a washing step with PBS for another 30 min. Afterwards, the bacterial suspension is pumped over the platelet layer for another 150 min. Changes in frequency and damping signals are recorded continuously throughout the experiment (Figure 1). In addition to the QCM measurements, the adhesion and interaction of platelets and bacteria were further visualized after the QCM run by scanning electron microscopy (Figure 2).
Conclusion

Bacterial adhesion to a platelet covered surface was successfully monitored by the qCell T instrument in real time. The adhesion of platelets on the sensor surface resulted in a frequency decrease and a dissipation increase upon mass adsorption, whereas the frequency signal increased again upon the adherence of bacteria.

Reference


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