



Assessment of primary hemostasis with an acoustic biosensor using shear dependent kinetics behavior: principle and limitations

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Preview for publications :

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Background and Objective:

Primary hemostasis involves in-flow interactions between platelets and sub-endothelial matrix at the wall of the damaged vessel. Assessing primary hemostasis defects would benefit from evaluation of the whole sequence of processes involved in platelet plug formation. We propose a novel label-free approach based on characterization of shear-dependent kinetics to evaluate the early stages of primary hemostasis. We developed a quartz crystal microbalance (QCM) biosensor to measure the amount of platelet deposited over time. With experiments and numerical simulations, we investigated the relevance of this approach and its limitations.

Methods:

We designed and built an acoustic biosensor based on a QCM whose gold surface was functionalized with Horm® collagen and used as the floor of a microfluidic chamber. We recorded with an impedance analyzer the variations of the QCM sensor resonance frequency during a 5-minutes perfusion through the chamber with anticoagulated whole blood from two healthy donors. The real-time QCM measurements performed at 500 - 1500/s range shear rate were supplemented with atomic force microscopy (AFM) observation at the end of the perfusion to evaluate the final morphology of the deposit and the surface coverage. Numerical simulations were used to understand the influence of deposit topology on the acoustic response.

Results:

For analyzing the complex kinetics profile of the frequency shift, we defined three metrics: total frequency shift, lag time, and growth rate. These metrics enabled the characterization of the kinetics of platelet deposition with good repeatability. We showed that these parameters measured at different shear rates, gave precise indications on the processes involved in the early stage of primary hemostasis, opening the way to analyze abnormal behavior. However we observed that the frequency shift was not always a direct measure of the platelet amount and depends on the surface topology of the deposit, which varies with the shear rate. The numerical simulation confirmed that if a platelet deposits is modeled as a structured viscoelastic load, the surface coverage affects the frequency shift of the sensor.

Conclusion:

Shear-dependent kinetics assays seems to be a promising method for studying primary hemostasis and its defects. We showed that QCM sensor measurements have to be combined with a precise evaluation of deposit topology to be fully usable.

Submitted Abstract

Instructions

- Submission of the abstracts according to [subject area and interdisciplinary area](#).
- The authors will have to tag each abstract with one subject area and up to three interdisciplinary area.
- The jury will decide on the abstracts to be presented as an Oral Communication or Poster presentation. **Only anonymized abstract submissions will be graded.** After acceptance, the authors will receive a notice from the organization / the scientific committee about the decision of acceptance by e-mail.
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Abstract topics

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- ☐ Antithrombotic treatment
- ☐ Arterial thromboembolism
- ☐ Coagulation and fibrinolysis
- ☐ COVID-19
- ☐ Crosstalks between hemostasis and other systems
- ☒ Diagnostics and laboratory tests
- ☐ Hereditary bleeding disorders
- ☐ In vitro and in vivo models of hemostasis
- ☐ Innovation and Novelties
- ☐ Platelets - Disorders of platelet function and numbers
- ☐ Platelets - Physiology
- ☐ Vascular wall biology and disorders

- ☐ Venous thromboembolism
- ☐ von Willebrand factor and ADAMTS13
- ☐ Miscellaneous

Interdisciplinary area

- ☐ Anesthesiology
- ☐ Angiology
- ☒ Basic research
- ☐ Cardiology
- ☐ Clinical practice
- ☒ Clinical research
- ☐ Gynecology/Obstetrics
- ☐ Hemato-oncology
- ☐ Hepatology/Gastroenterology
- ☐ Intensive care
- ☒ Laboratory medicine
- ☐ Laboratory technology
- ☐ Neurology
- ☐ Pediatrics
- ☐ Transfusion medicine
- ☐ Trauma/Surgery

Abstract

Title

Assessment of primary haemostasis with an acoustic biosensor using shear dependent kinetics behavior: principle and limitations

Background and Objective

Primary haemostasis is a complex dynamic process, which involves in-flow interactions between platelets and sub-endothelial matrix at the area of the damaged vessel wall. The diagnosis of primary haemostasis defect would benefit from evaluation of the whole sequence of mechanisms involved in platelet plug formation. This work proposes a new approach that is based on characterization of the shear-dependent kinetics that enables the evaluation of the early stages of primary haemostasis. We propose to use a label-free method with a quartz crystal microbalance (QCM) biosensor to measure the platelet deposits over time onto covalently immobilized type I fibrillar collagen. Using experiments and numerical simulations, we investigate the relevance of the approach and its limitations.

Methods

We designed and built an acoustic biosensor based on a QCM sensor placed at the bottom of a microfluidic chamber. The chamber was designed to obtain wall shear rates in a range of 500 -

1500s⁻¹ with whole blood flow. The QCM gold surface was functionalized to immobilize Horm® collagen by its primary amines at room temperature overnight. The biointerface was then blocked with 0.1% of BSA in sodium acetate buffer solution 10mM pH 5.5 to prevent non-specific binding.

We recorded the variations of the QCM sensor resonance frequency during five minutes perfusion with anticoagulated whole blood with an impedance analyzer. In the experiments, we used the whole blood of two healthy donors, testing shear rate in the 500 - 1500s⁻¹ range. The real-time QCM measurements were completed with atomic force microscopy (AFM) observation of the deposit at the end of the perfusion to evaluate the final morphology of the deposit and its surface coverage.

Numerical simulations were used to understand the behavior of the system and particularly the influence of surface coverage on the acoustic response.

Results

For analyzing the complex kinetics profile of the frequency shift we defined three metrics: total frequency shift, lag time, and growth rate. These metrics allowed to characterize the complex kinetics of the platelet deposit for the different shear rates with good repeatability. We show that these parameters measured at different shear rate, give a precise picture of the different mechanisms involved in the early stage of primary haemostasis, opening the way to analyze pathological behavior.

However we observed that the frequency response was not always a direct measure of the platelet amount and depends on the surface coverage of the deposit, a topology that varied with the shear rate. The numerical simulation confirmed that if the platelet deposits is modelled as a structured viscoelastic loads, the surface coverage affects the frequency shift of the sensor.

Conclusion

Shear-dependent kinetics assays seems to be a promising advanced method for screening of primary haemostasis. However, the use of QCM sensor alone is not sufficient to evaluate accurately the amount of deposited platelet and it has to be combined with a separate evaluation of the deposit surface coverage.

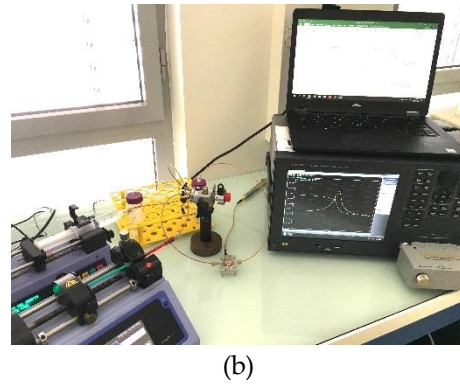
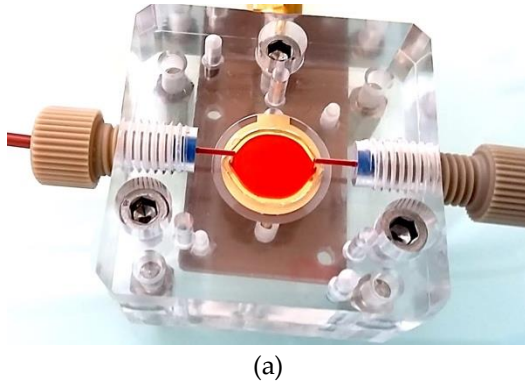


Fig. 1. Photograph of the whole blood perfusion chamber with an installed QCM biosensor (a) and the experimental setup (b)

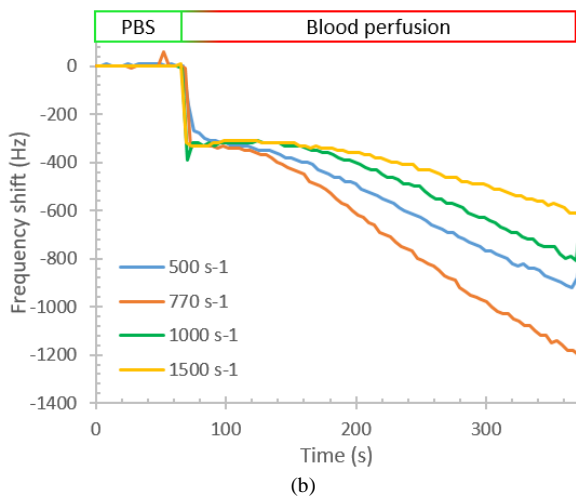
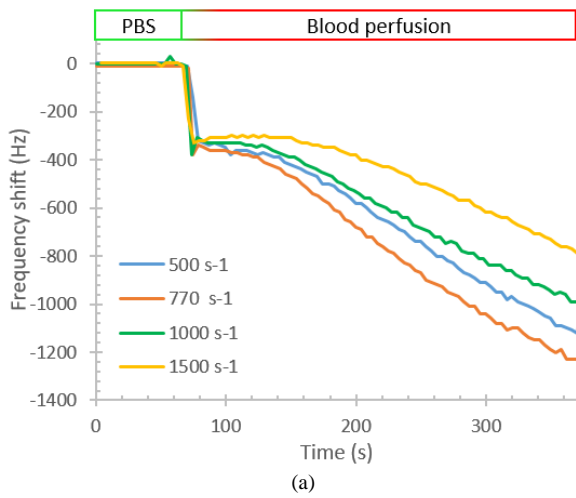


Fig. 2 QCM sensor admittance frequency shift (Hz) recorded during the blood perfusion during five minutes for two donors (donor1 (a) and donor2 (b)) at a shear rate of 500s⁻¹ (blue), 770s⁻¹ (orange), 1000s⁻¹ (green) and 1500s⁻¹ (yellow).

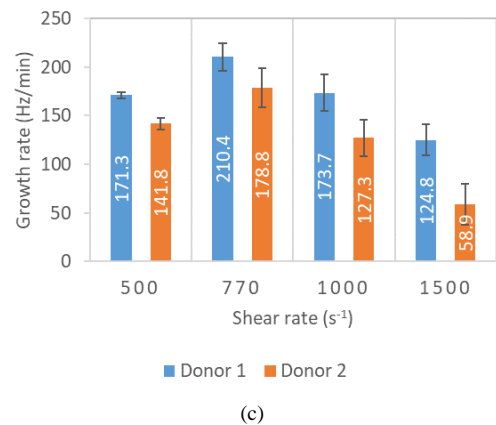
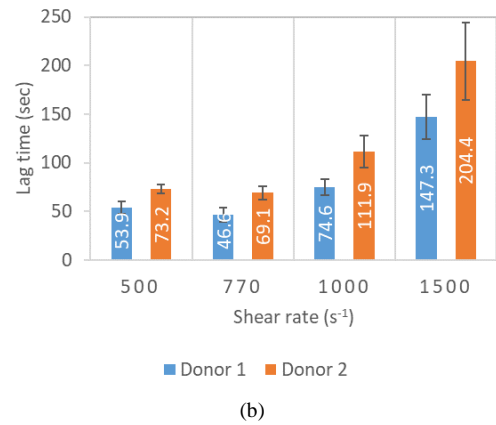
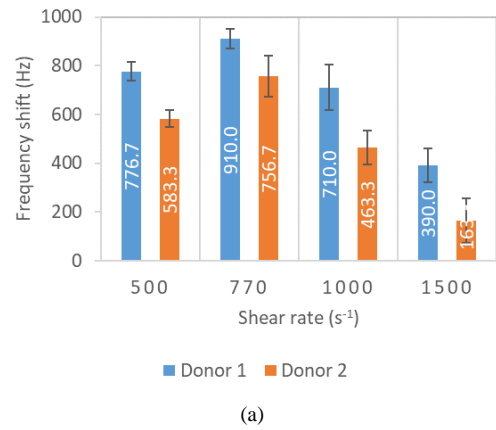


Fig. 3. Bar charts of the three defined metrics as a function of shear rates for two healthy donors: TFS (a); lag time (b); growth rate (c). Error bars show the absolute deviation from the mean value at the same shear rate the tests were made for the same donor on three different days.

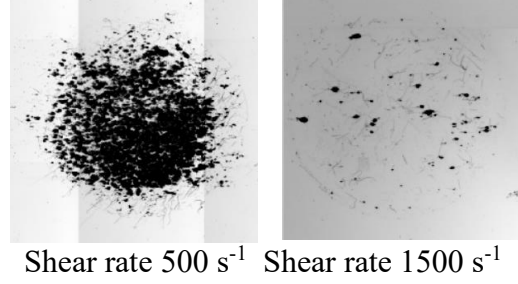
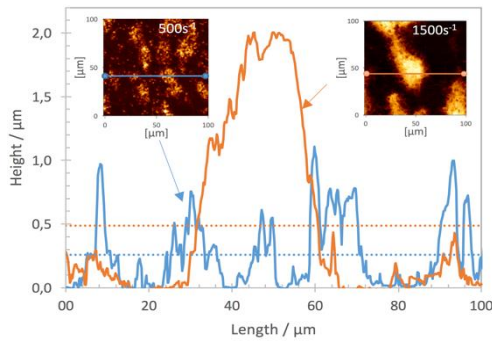


Figure 4. (left) Height profile of platelet deposits across the biointerface for shear rates 500s⁻¹ (blue curve) and 1500s⁻¹ (orange curve) (average height for 500s⁻¹ (blue) and 1500s⁻¹ (orange) are shown with dotted lines). Insets: AFM images (height trace) of platelet deposits obtained at 500s⁻¹ (on the left) and 1500s⁻¹ (on the right) shear rates respectively. (right) Optical microscopy image of platelets deposits on collagen biointerface at 500s⁻¹ (on the left) and 1500s⁻¹ (on the right).

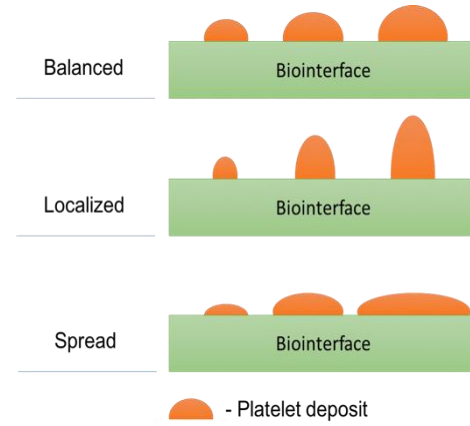
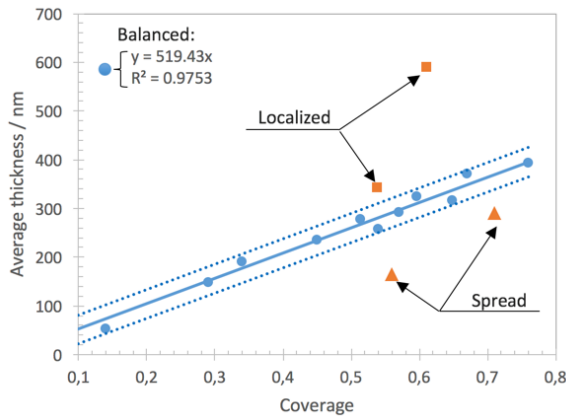
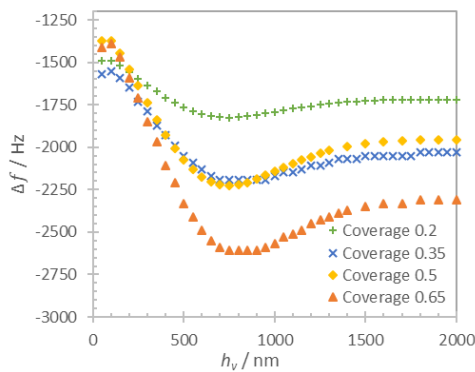
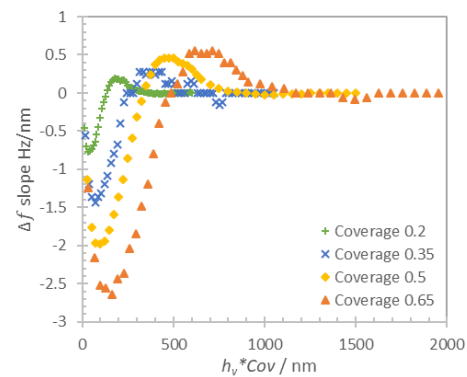


Figure 5. Platelet deposits average thickness versus deposits coverage. (left) Blue dots show the cases that are found within one standard deviation (dashed lines) of the linear regression (solid line). Orange dots correspond to the cases with deviation from linear regression larger than one standard deviation. Linear regression (solid line) and corresponding coefficient of determination R^2 are shown for the data points within one standard deviation (blue dots). (right) Schematic illustration of the deposits growth for the "balanced", "localized" and "spread" cases.



(a)



(b)

Figure 6. Computation results for acoustic resonator loading with structured viscoelastic layer of period 100 nm with the coverage in a range 0.2-0.65 and a range of thicknesses 50-2000 nm. Frequency shift versus structure thickness (a) and slope of frequency shift versus thickness (h_v) multiplied by coverage (Cov) for each structured layer (b). The frequency shift is calculated relative to the unloaded resonator frequency in air