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Oral or Poster

MULTIPLEXED BIOSENSOR USING QUARTZ-ON-SILICON MICRO-ACOUSTIC (QSIM) TECHNOLOGY FOR IN-VITRO LABEL-FREE INVESTIGATION OF HEMOSTASIS

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We report on the development of a new type of micro-acoustic biosensor that combines compactness and ease of use of microsystems and performance of monocrystalline quartz biosensors for medical diagnosis. The biosensor was used for the assessment of primary hemostasis, performing real-time analysis of platelet plug formation kinetics using whole blood.

Resonant acoustic sensing using quartz crystal microbalances (QCM) is a recognized technology for biological interaction analysis (ligand-protein or protein-protein)[1,2]. QCM show high quality factor (Q factor) in liquid, high sensitivity[3], robustness and low cost. However, they lack integration capabilities to build multiplexed biosensor with multi-target analysis of biological samples (Fig.1). Surface acoustic wave or thin-film piezoelectric based devices have straightforward integration and multiplexing ability, but they are still unable to compete with QCM, mostly due to low Q factor in liquids and high fabrication costs. We propose a new Quartz-on-Silicon Micro-acoustic (QSiM) technology (Fig.2), relying on standard quartz crystal and silicon wafer technology, making integration cost effective, while keeping the shear wave resonance of the QCM in a quartz membrane for high sensitivity[4]. Actually, the QSiM technology keeps the Q factor in water above 1000 by implementing acoustic reflectors (Fig.2-inset) that efficiently localize elastic vibration within the sensing unit and reduce anchor dissipation losses (Fig.3-left). The reflectors also enable dense integration of sensing units without cross-talk, suitable for multiplexed sensor (Fig.3-right).

We built the biosensor for primary hemostasis assessment by placing a 3 sensing units chip inside a parallel plate perfusion chamber machined in PMMA (Fig.4-left), and connecting the sensing units to a multi-channel impedance analyzer module developed using electronics from OpenQCM (Fig.4-right). The sensing units gold top surfaces were individually grafted with different biointerfaces. Each experiment consisted in letting whole blood flow on the chip surface for 10 min at a shear rate of $1500s^{-1}$ to comply with recommendations for the analysis of primary hemostasis[5]. Anticoagulated whole blood was used within 2 hours after blood collection from healthy donors. During the infusion, we recorded changes in impedance of each units and extracted resonant frequency shift (linked to mass of deposited biological material) and loss factor evolution (linked to viscoelastic properties of deposited material).

Using biointerfaces grafted with fibrillary Horm collagen or passivated with BSA, we first verified the good repeatability and the low cross-sensitivity between neighboring sensing units of the sensor. Then, in order to demonstrate the ability of the biosensor to perform simultaneous analysis of multiple factors involved in primary hemostasis, we grafted the three biointerfaces with, respectively, a-vWF antibody, a-fibrinogen antibody and Horm collagen (Fig.6). The experiment depicted in Fig.7 (acoustic signature confirmed by optical images) reveals the expected presence of platelets deposition on the a—vWF and collagen biointerfaces. Regarding the a-fibrinogen biointerface, a much lower signal shows that only fibrinogen capture is detected, since non-activated platelets cannot interact with fibrinogen at this shear rate.

The developed QSiM bioassay technology has the potential for broad applications in biomedical field as an improved alternative to quartz crystal microbalances based devices for multiplexed integrated bio-interactions analysis.

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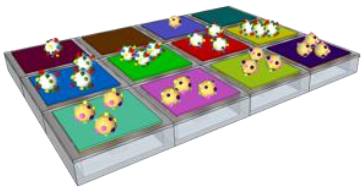

Figure 1: Principle of multiplexed label-free biosensor with 12 sensing units for multiple bio interaction analysis.

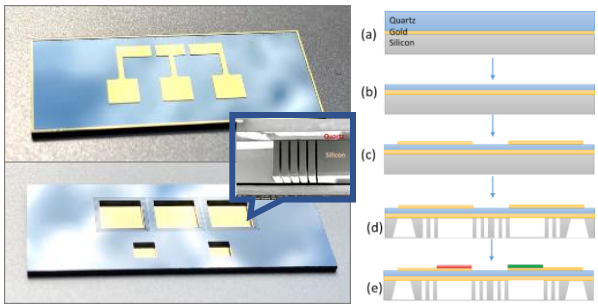

Figure 2: (left) Front and back view of a 3 sensing units (in line) chip (inset) SEM view of reflector at the edge of the sensing unit membrane (right) Process flow chart with Quartz–on-Si wafer showing 2 sensing units.

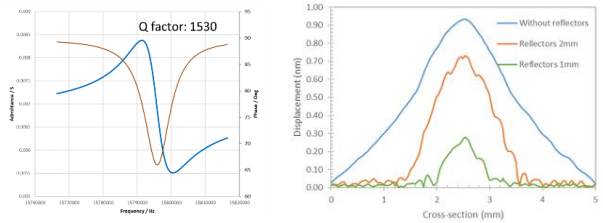

Figure 3: (left) Impedance measurement showing a Q factor of 1350 in water (right) Acoustic wave amplitude at sensing unit surface for membrane (blue) without reflector or with reflectors spaced by (red) 2mm or (green) 1mm.

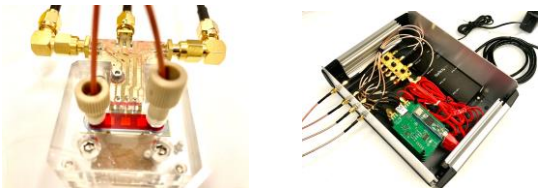

Figure 4: (left) PMMA microfluidic chamber with the 3 sensing units chip and electric readout circuit (right) 3 channel modular electronic for impedance measurement

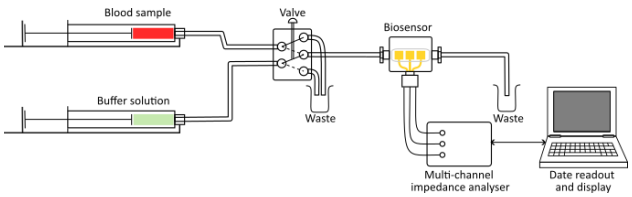

Figure 5: Experimental set-up for primary hemostasis test with whole blood (we inject PBS before and after the 10 min perfusion of whole blood with anticoagulant)

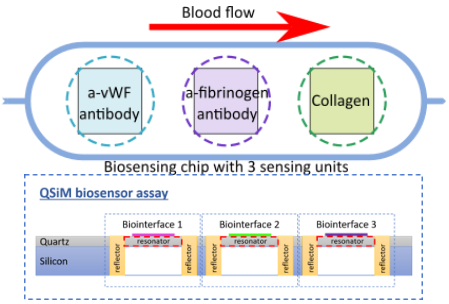

Figure 6: Top and cut-out schematic of biosensor with 3 sensing units with different biointerfaces (a-vWF, a-fibrinogen, HORM collagen) for primary hemostasis test.

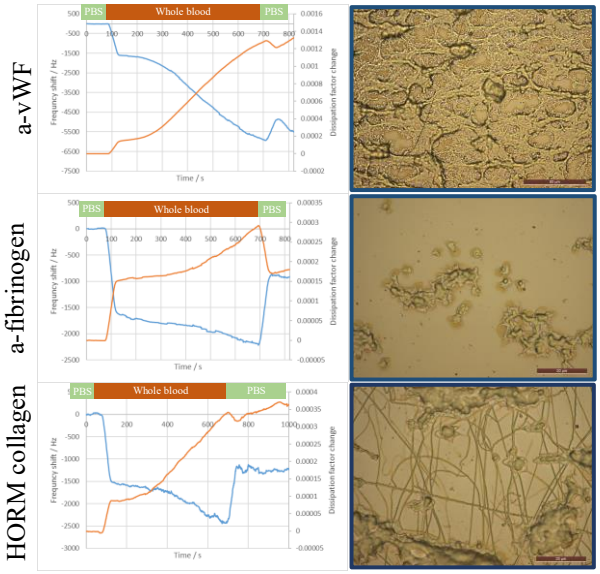

Figure 7: (left) Real-time evolution of the frequency shift (blue) and loss factor (red) for the 3 sensing units. (right) optical image of each sensing unit surface at the end of the 10 min whole blood perfusion and PBS rinsing.

1. L. F. Harris and A. J. Killard, “Microfluidics in coagulation monitoring devices: a mini review,” Analytical Methods, vol. 10, p. 3714–3719, 2018
2. A. Oseev, et al., "Assessment of Shear-Dependent Kinetics of Primary Haemostasis with a Microfluidic Acoustic Biosensor," IEEE Trans. on Biomed. Eng., vol. 68, p. 2329–2338, 2021
3. Y. Q. Fu, et al., “Advances in piezoelectric thin films for acoustic biosensors, acoustofluidics and lab-on-chip applications,” Progress in Materials Science, vol. 89, p. 31–91, August 2017.
4. A. Oseev et al, "Acoustic biosensor assay assembly". Europe Pat. EP4016074A1, 2022.
5. R. V. Kruchten, et al., "Measurement of whole blood thrombus formation using parallel-plate flow chambers – a practical guide," Platelets, vol. 23, p. 229–242, April 2012