A HIGH FUNDAMENTAL FREQUENCY QUARTZ CRYSTAL BIOSENSOR INTEGRATED INTO AN ELECTRO-WETTING-ON-DIELECTRICS BASED LAB-ON-A-CHIP

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ABSTRACT

We demonstrate the operation of an Electro Wetting on Dielectrics (EWOD) hybrid lab-on-a-chip system by utilizing a Quartz Crystal Microbalance (QCM) resonator as mass-sensitive sensor. We have tested the formation of a phospholipid monolayer out of an aqueous buffer suspension onto the integrated sensor. The altered mass load resulted in a shift of the resonance frequencies. Subsequently, the formation of a protein multilayer was monitored by QCM frequency and contact angle measurements. Using these sample applications, we were able to demonstrate and verify the feasibility of our prototype combining a mass-sensitive QCM sensor with digital microfluidics based on EWOD.

KEYWORDS: Quartz Crystal Microbalance, QCM, Electro Wetting on Dielectrics, EWOD, bio molecules

INTRODUCTION

The realization of a lab-on-a-chip platform, which is designed to perform and detect (bio-)chemical reactions by directing micro liter volumes of different reagents and reaction products, is one of the major goals within microfluidics. Digital microfluidics deals with discrete droplets which are individually addressed and actuated and holds the advantage of a high degree of freedom and flexibility to react on real-time measurement results. The realization of the single droplet actuation affects all design requirements for the integration of further microfluidic components and sensor elements.

The intention of our contribution is to show the feasibility of integrating a Quartz Crystal Microbalance (QCM) into a digital microfluidics platform utilizing Electro Wetting on Dielectrics (EWOD) as actuation mechanism. The QCM is a mass-sensitive sensor which is (in a functionalized condition) able to indicate the binding of bio-molecules by a shift of the resonance frequency [1].

THEORY

Electro Wetting on Dielectrics can be used for manipulations (creation, transport, mixing and divisions) and accurate handling of multiple small volume droplets [2, 3, 4] on a common electrode array. Different (bio)-chemical reactions can be performed on the same chip by simply changing the sequence of droplet manipulations.

Different contributions to the total energy, e.g., electric potential differences, surfactants as well as gravitation change the actual droplet shape [5]. Applying a voltage \( V \) between two electrodes, which are electrically isolated from the droplet by a dielectric layer, changes the charge distribution at the droplet surface, and lowers the effective surface energy of the solid/liquid interface \( \gamma_{SL} \) proportional to the specific capacitance \( c \) of the isolating layer, described by the Lippmann-Young equation:

\[
\gamma_{SL}(V) = \gamma_{SL} - \frac{cV^2}{2}
\]

As a result a pulling force towards the energized electrode and a consecutive transportation, see Fig. 1, is achieved.

As a sensor a QCM resonator with a thickness shear mode resonance frequency of \( f_0 = 50 \text{MHz} \) was utilized. A change in mass load per unit area \( \Delta m \) is the main detectable effect of biochemical binding interactions under study. According to the Sauerbrey relation [6], a change in surface mass deposition, originating, e.g., from biochemical binding to an interface film on the sensor surface, results in a corresponding shift of the fundamental resonance frequency \( \Delta f \). The Sauerb-
The relationship is given below in (3), where \( \rho \) denotes the mass density (2,648 g/cm\(^3\)) and \( \mu \) the shear modulus (29,47 GPa) of the QCM. A crucial factor for the performance of QCM systems is the fundamental resonance frequency, \( f_0 \), of the piezoelectric sensor due to the quadratic dependency of frequency shift \( \Delta f = -2f_0^2 \frac{4}{\rho \mu} \frac{1}{h} \). Following the resonance condition the fabrication of thickness-shear-mode resonators with higher values of \( f_0 \) is basically achieved by reducing the plate thickness \( h \) of the sensor [1]. An approach to realize higher fundamental frequencies is to utilize quartz resonators with an “inverted mesa” structure. These quartz disks with a diameter and thickness of about 5 mm and 0.1 mm, respectively, have a membrane of reduced thickness only in their small central circular area of about 5 mm\(^2\), which results in a good mechanical stability provided by the surrounding thicker material.

**EXPERIMENTAL**

The integration of a QCM sensor into an EWOD platform requires adjustments to the electrode design and the applied coating layers. The QCM was bonded as planar as possible into the EWOD platform, in our case into the base plate; see Fig. 2. For a reliable EWOD actuation the coating of all surfaces contacting the droplet has to be hydrophobic with a low hysteresis angle. This was realized by a monolayer (SAM) of 1-octadecanethiol (Sigma Aldrich) self assembled on a PVD deposited gold layer, which allows for the formation of a phosphor-lipid monolayer out of an aqueous buffer suspension, which serves as a sample reaction for bio-chemical bindings in general. The functionalized surface shows hydrophobic properties with a contact angle of 101±1° and a low hysteresis angle \( \alpha \) (\( \alpha \) represents the “pinning” of the contact line up to a certain unbalance of the surface tensions).

A solution of phosphor-lipids which carried biotin residues on 20% of the phospholipid head groups in Phosphate-buffered saline (PBS) were prepared as described in [1] and single droplets thereof were EWOD actuated to the QCM.

**RESULTS AND DISCUSSION**

The consecutive and reversible binding of the sequence Phospholipids on a SAM of 1-octadecanethiol and Streptavidin on biotin carrying phospholipids was shown in [1]. In that contribution, the reagents were provided in a continuous flow whereas in our contribution the sample liquid is provided to the QCM sensor element in terms of single droplets, which allows for an individual control and manipulation prior and posterior to the measurement. A series of phosphate buffered saline (PBS) droplets of 10 μl volume were transported to and away from the QCM sensor and initial reference spectra were taken at ambient temperature of 24.1°C to determine the fundamental frequency and corresponding quality factor \( Q = f_0 / \Delta f_{FWHM} \) (50,777 MHz and 394, respectively) without an additional mass load. Subsequently a series of droplets containing biotin carrying phosphor lipids was directed over the QCM sensor. In agreement with theory, a shift to lower resonance frequencies of the QCM was determined via impedance measurements and a stable level was reached after 8 minutes indicating the formation of a phospholipid monolayer, which carries biotin residues on 20% of the phospholipid head groups [7]. Measurements on PBS confirmed the stability of the formed monolayer. A multilayer formation was achieved by directing a series of streptavidin enriched droplets, which allows for bio-specific binding to the Biotin-presenting phospholipid coating of the QCM [8]. An additional decrease of ~20 kHz in the resonance frequency of the QCM was determined; see Fig 4 (left). After the first three measurements in a time interval of 3 minutes the signal did not decrease further. Terminatory, two PBS droplets were placed on top of the sensor to confirm the multilayer formation as the origin of the frequency shift and to rule out shifts due to a different viscosity of the dissolution.
In a second experiment we brought a droplet detergent of octyl-D-glucopyranoside to the surface coated with phospholipids. The binding tail was removed and the original resonance frequency restored. After the measurements, which indicated the presence of one of both substances, we tried to direct the droplets away from the sensor to use them for further reactions. In the case of a positive detection of bindings the hydrophobic properties of the functionalized QCM get lost. Thus, we were not able to drag the droplet away from the QCM by means of electro wetting actuation.

As a last test to quantify the decrease of the hydrophobicity we measured the contact angle of PBS droplets containing phospholipids. A continuous drop of 12° in total over 10 min was found, see Fig. 4 (right) indicating the stochastic unspecific binding of phospholipids onto the hydrophobic surface. The approaches of either washing away the binding molecules or the new formation of a hydrophobic surface are most promising to prevent the sticking of the droplet.

CONCLUSION

We presented the integration of a functionalized QCM sensor with high fundamental frequency into a platform designed for EWOD actuations. The shift of the resonance frequency was detected, indicating the binding of bio-molecules out of single droplets to the functionalized surface. Thus, the applicability of functionalized QCM sensors in digital microfluidics was shown. The drawback of this detection method is that after the first binding of lipids the hydrophobic layer gets more and more hydrophilic. Therefore, the QCM sensor principle has to be applied to a different bio-chemical system where the hydrophobic properties are conserved during the detection process. Currently, we are investigating chemical reactions which restore the original or form a new hydrophobic layer.

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REFERENCES


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