Film Bulk Acoustic Resonators of High Quality Factors in Liquid Environments for Biosensing Applications

by

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Micro-electro-mechanical systems (MEMS) film bulk acoustic resonator (FBAR) demonstrates label-free biosensing capabilities and is considered to be a promising alternative of quartz crystal microbalance (QCM). FBARs achieve great success in vacuum, or in the air, but find limited applications in liquid media because squeeze damping significantly degrades quality factor \((Q)\) and results in poor frequency resolution. A transmission-line model shows that by confining the liquid in a thickness comparable to the acoustic wavelength of the resonator, \(Q\) can be considerably improved. The devices exhibit damped oscillatory patterns of \(Q\) as the liquid thickness varies. \(Q\) assumes its maxima and minima when the channel thickness is an odd and even multiple of the quarter-wavelength of the resonance, respectively. Microfluidic channels are integrated with longitudinal-mode FBARs (L-FBARs) to realize this design; a tenfold improvement of \(Q\) over fully-immersed devices is experimentally verified. Microfluidic integrated FBAR sensors have been demonstrated for detecting protein binding in liquid and monitoring the Vroman effect (the competitive protein adsorption behavior), showing their potential as a promising bio-analytical tool. A contour-mode FBAR (C-FBAR) is developed to further improve \(Q\) and to alleviate the need for complex integration of microfluidic channels. The C-FBAR consists of a suspended piezoelectric ring made of aluminum nitride and is excited in the fundamental radial-extensional mode. By replacing the squeeze damping with shear damping, high \(Qs\) (189 in water and 77 in human whole blood) are obtained in semi-infinite depth liquids. The C-FBAR sensors are characterized by aptamer
- thrombin binding pairs and aqueous glycerine solutions for mass and viscosity sensing schemes, respectively. The C-FBAR sensor demonstrates accurate viscosity measurement from 1 to 10 centipoise, and can be deployed to monitor \textit{in-vitro} blood coagulation processes in real time. Results show that its resonant frequency decreases as the viscosity of the blood increases during the fibrin generation process after the coagulation cascade. The coagulation time and the start/end of the fibrin generation are quantitatively determined, showing the C-FBAR can be a low-cost, portable yet reliable tool for hemostasis diagnostics.
DEDICATION

To My Parents
ACKNOWLEDGMENTS

It is difficult to overstate my gratitude to my advisor, Dr. Junseok Chae. He gave me the opportunity of doing my thesis research under his advice and made this work possible and brilliant by providing the best resources and academic advices a student could ask for. His contagious optimism and enthusiasm were a constant source of motivation for me. Over the past years, Dr. Chae helped me grow both professionally and personally. It has been a privilege for me to work under his supervision.

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Chapter 1

INTRODUCTION

1.1 Background

Selective and sensitive detection of biomolecules is a powerful analytical technique in modern molecular biology (Stagni et al, 2007; Carrara et al, 2009; Berggren et al, 1999). Among the various types of sensing technologies, fluorescent and radioactive labeling techniques provide direct measurement and extremely high sensitivities. However, the labeling process which may introduce chemical modifications to the target molecules is not always desirable (Burg et al, 2006).

Label-free biomolecule sensors often measure the changes in the physical properties, such as electrical charge, optical refractive index, mechanical surface stress, or accumulated molecular mass. To provide selectivity of the target molecules, the sensor surface is typically immobilized with receptors which preferentially bind the molecules of interest. The properties of the surface are altered when the binding events occur and a signal is generated. This scheme does not require a secondary antibody or fluorescent labeling of the target molecules, thus enables detection of which labeling would interfere with the binding reaction or in which real-time measurements of the binding kinetics are of interest (Burg et al, 2006). The label-free sensors also deliver fast response and provide quantified data as they directly measure the changes of the physical properties at the sensor surface where the immobilized receptors bind the target molecules. Surface plasmon resonance (SPR) (Brecht and Gauglitz 1997) and quartz crystal
microbalance (QCM) based instruments are currently the two most widely used methods for label-free biomolecular interaction analysis, with applications ranging from fundamental research in systems biology to drug discovery and quality control. However, it is difficult to microfabricate SPR and QCM in batch to miniaturize them and lower the manufacturing cost, sometimes limiting their applications.

Micro-electrical-mechanical system (MEMS) transducers enable the detection of biomolecules in microfluidic systems with extremely small sample volumes. The integration of microfluidic sample into lab-on-a-chip sensors can leverage experimental efforts in system biology and pharmaceutical research by increasing analysis throughput while dramatically reducing reagent cost. MEMS transducers also inherit the advantages of modern integrated circuits (IC): batch production, mass manufacturing, and small size, which directly relate to lowering costs, good reproducibility in dimensions as well as in material properties and performance, and versatility. Therefore MEMS is a promising candidate for miniaturized detection systems with real-time monitoring capabilities for point-of-use and disposable applications.

Generally, there are three classes of MEMS bio-chemistry transducers: electronic, optical and mechanical transducers. Each of them has specific advantages and its own limitations. Electronic field-effect sensors can be highly sensitive to charged molecules regardless of molecular weight, but they usually require low ionic strength solutions and tight binding of ligands to the surface due
to the charge screen effect (Fritz et al, 2002; Cui et al, 2001). SRP, one of attractive optical sensors, measures the refractive index in the evanescent field of planar waveguides, which limits the thickness of the sensitive layer to less than 100 nm and requires precise alignment of external optical components to the microfabricated device (Voros et al, 2002; Lukosz 1995; Cooper et al, 2002). Surface stress sensors are generally featured with simple configuration and high sensitivity in certain assays. However, the relationship of surface stress and the density of bound targets depends on various physical properties of the interface, including electrical charge, hydrophobicity, and steric hindrance between adsorbed molecules; therefore the quantitative analysis using such method is rather limited. Additionally, the sensor surface may require different functionality treatment from one target molecule to the other, which complicates the assay development (Fritz et al, 2000; Wu et al, 2001; Savran et al, 2003).

Another class of micromechanical transducers is formed by mechanical or acoustic resonators of which the shift of the natural resonant frequency provides a direct quantitative measure of the mass adsorbed to the resonator surface. Micro-/nano-cantilever resonators have achieved great success for biochemical sensing in vacuum or gaseous environments; samples as light as 7 zepto-grams can be weighed in vacuum (Thundat et al, 1995; Lange et al, 2002; Gupta et al, 2004; Yang et al, 2006) where a single proton level resolution is within reach. However, while the sensor is in contact with liquids, the mass sensitivity and frequency resolution of the resonant sensors severely degrade due to the low quality factor ($Q$) and large effective mass induced by the viscous drag, thus preventing many
applications in life/medical sciences where sensors need to operate in liquid environments. Although some interesting designs possess potentials to minimize the viscous loss and alleviate the performance degradation, either the sensitivity is still far inferior to the devices in the air or vacuum (Weinberg et al, 2003; Zhang and Kim 2005; Braun et al, 2005; Pang et al, 2006) or significant fabrication complexity, thus cost, is required (Burg and Manalis, 2003; Burg et al, 2006).

Film bulk acoustic resonator (FBAR) is one type of acoustic resonators. Featuring with a piezoelectric thin film sandwiched between two metal electrodes, the mechanical vibration excited at a specific frequency generates an acoustic standing wave due to the acoustic isolation from the surrounding medium. Because of the small film thickness, ranging from several micrometers down to tenth of micrometers, FBARs possess resonant frequencies from approximately 100 MHz up to 10 GHz. Combining with the high intrinsic $Q$ in the air, FBAR initially finds applications in the wireless communication circuits, such as radio frequency (RF) filters and duplexers (Lakin and Wang 1981; Lakin et al, 1993; Mueller 2001), partially replacing an earlier technology based on surface acoustic wave (SAW) devices, due to the low loss and high power handling capabilities of the FBARs. FBAR can also be considered as a miniaturized version of QCM but can be exited in a different mode; therefore FBARs have attracted intense interests for bio/chemical sensors (Yan et al, 2007; Bender and Krim 2005; Zhang et al, 2005). The driving force of this development has been to replace the use of expensive single crystalline substrate in the ~MHz range with piezoelectric thin films which would provide significantly higher frequencies (~GHz) and
integration capability with IC to reduce manufacturing cost. FBAR has a resonant frequency ($f_r$) mainly determined by the structure and thickness of the device. As the magnitude of the frequency shift is proportional to the attached mass, FBARs can be used to detect the captivation of target analytes and therefore provide a quantitative measurement of the biological/chemical process. A typical ZnO based FBAR sensor has a thickness of ~1 µm of the piezoelectric resonant composite, possess a significantly higher frequency (up to 2 GHz) than QCMs, offering a mass sensitivity of more than 1000 Hz cm$^2$/ng, which is over 1000 times higher than that of typical QCMs (Bender and Krim 2005; Gabl et al, 2004; Yan et al, 2005; Lee et al, 2002).

FBAR sensors have been highly successful for mass sensing in the air or vacuum due to their high $Q$s in the low damping gaseous environments. $Q$ of up to 2000 has been reported in the air (Yan et al, 2005; Zhang and Kim 2005). Consequently, as the frequency resolution, defined as the detection limit of the frequency shift $\Delta f$, is practically proportional to $Q^{-1}$ of the resonator (Ikehara et al, 2007), FBAR sensors in the air allow very fine frequency resolutions, thus mass resolutions. However, in liquids, FBAR encountered similar challenges as other mechanical resonators, such as micro-cantilevers. $Q$ decreases severely. The previously reported $Q$ value of 15 in water indicates an up to 20 times worse minimum detectable frequency shift and a 95% reduction in corresponding mass resolution compared to that of FBARs operating in the air (Zhang and Kim, 2005). Several attempts have been reported to address the limitation. “Dip and dry” method has been employed to measure the resonant frequency in air before
and after the device has been exposed to the sample (O’sullivan and Guilbault 1999; Ilic et al, 2004). This technique does not allow real-time measurements for studying binding kinetics and the reliability can hardly be ensured because of the potential contamination. Another attempt replaces the traditional out-of-plane (longitudinal mode) FBARs with in-plane (shear mode) FBARs for in-liquid sensing (Link et al, 2007; Wingqvist et al, 2007; Weber et al, 2006). Shear mode FBARs experience less viscous drag from the surrounding liquid and consequently alleviate the degradation of the $Q$ and mass resolution. Typical $Q$s of 100–150 have been reported in water for shear mode FBARs. However, the shear mode FBARs compromise sensitivity to be only one-third that of a longitudinal mode FBAR (L-FBAR) with similar dimensions (800 versus 2500 Hz·cm$^2$/ng) (Link et al, 2007; Wingqvist et al, 2007).

Other than the mass detection scheme, FBARs, in a modified form, can also be sensitive to the viscosity of the surrounding liquids which enables them to be an effective viscosity sensor and to be potentially deployed for specific clinical applications.

Coagulation of whole blood provides important information for the medical diagnosis of hemostasis disorders and clinical treatment/surgical procedure for cardiovascular diseases (Whitmore 1968; Gross and Hwang, 1981). The blood coagulation process can be described as an enzymatic cascade, activated in two cases: immediately after the damage of any blood vessel as the intrinsic system or if blood contacts with foreign materials as an extrinsic system
Numerous monitoring methods exist to assess the coagulation process. The most commonly used ones include: the activated partial thromboplastin time (aPTT) test measures the contact factors in plasma which initiated the intrinsic pathway; prothrombin time (PT) test monitors the released tissue factors which initiated the extrinsic pathway (Reverdiau-Moalic et al, 1996); fibrinogen testing generally uses Von Clauss method to measure the amount of fibrinogen presented in blood (Palareti et al, 1991); platelet count determines the platelet number and enzyme immunoassay (EIA) or equivalent methods measure the released thrombospondin (TSP) to determines the degree of activity for the platelets (Bergseth et al, 2000). The kinetics of blood coagulation and clot characterization are studied using various metrology equipments, such as spectrophotometry (Sanchez et al, 2002), ellipsometry (Walivaara et al 1996), surface plasmon resonance (SPR) (Hansson et al, 2002; Vikinge et al, 2000) and free oscillation rheometry (Hansson et al, 2002). Acoustic sensors, emphasizing QCM (Bandey et al, 2004; Andersson et al, 2005; Muller et al, 2010), measure viscosity of a blood sample which is very sensitive to the conversion process from fibrinogen to fibrin and, consequently, are suitable for the real time measurement of the coagulation process. These devices operate in resonance modes of pure thickness shear polarization, of which the resonant frequency is coupled with the blood viscosity as the resonant frequency changes with the square root of the viscosity density product. Acoustic biosensors can provide valuable blood rheology information for controlling the coagulation system during surgical operations or after mechanical heart valve replacement (Bandey et al, 2004), and
feature several advantages, including fast and real-time electronic read-out, robust structures, and inexpensive manufacturing process.

A modified FBAR in topology is excited in a contour mode other than the longitudinal mode in traditional FBARs. The contour-mode FBAR (C-FBAR) is found to be sensitive to both surface mass and viscosity change of coupled liquids, which allows it to be viscosity sensor and a blood coagulation monitoring device. Comparing to QCM, C-FBAR is considerably small in form factor, low in manufacturing cost, compatible with CMOS batch fabrication, and can handle extremely minute sample volumes, therefore they have best potentials for field applications and disposable devices.

1.2 Device concept

The primary feature that sets this work apart from research in the area of FBAR sensors in liquid is that we significantly increase the $Q$ of FBARs in liquid, by a factor of at least 12, and allows real-time measurement of bio/medical events, either protein binding or whole blood coagulation. To achieve this goal, two types of FBARs are developed in this work.

When a L-FBAR is in contact with liquid, a substantial acoustic intensity generated by the resonator transmits into the liquid and attenuates in the lossy media. However, if the liquid is confined to a thin layer of which the thickness is comparable to the acoustic wavelength the incident acoustic wave reflects at the liquid/enclosure interface and the acoustic energy is well entrapped in the resonator body. In this work, we developed a L-FBAR integrated with a microfluidic channel, of which $Q$ of up to 150 has been experimentally achieved,
an order of magnitude higher than the state-of-the-art MEMS sensors. Figure 1.1 shows a schematic of the L-FBAR integrated with a microfluidic channel.

![Figure 1.1 Schematic structural figure of a MEMS L-FBAR (longitudinal-mode film bulk acoustic resonator) sensor integrated with a microfluidic channel.](image)

The C-FBAR removes the complexity of the integrated microfluidic channel and further improves the $Q$. As shown in Figure 1.2, the C-FBAR consists of a ring-shaped piezoelectric AlN resonator connected to the silicon substrate with one or multiple anchors. The resonator is excited in a pure radial-extensional mode, while the liquid droplet is directly dispensed above the resonator body. By replacing the squeeze damping with shear damping, the acoustic energy loss is alleviated. $Q$ of up to 189 has been successfully achieved in water.

In this dissertation, we propose, develop, and characterize the two types of high-$Q$ FBARs in liquids and deploy them for biosensing applications, which include the detection of protein binding and monitor of blood coagulation $in vitro$. 

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Chapter 2 proposes the L-FBAR with integrated microfluidic channels. The operating principle, fabrication techniques are described. The $Q$ of such devices is theoretically predicted by a transmission-line model and is experimentally characterized and verified. Chapter 3 presents the implementation of an oscillator using the high-$Q$ L-FBAR in liquids. Protein adsorption behaviors at the sensor surface are investigated. Chapter 4 discusses the C-FBAR. The modeling, fabrication, and characterization are addressed. The C-FBAR as a viscosity sensor is further used to monitor the blood coagulation in vitro in real-time. Lastly, Chapter 5 concludes this work and suggests the future work.

![Figure 1.2 Schematic of a MEMS C-FBAR (contour-mode film bulk acoustic resonator) sensor with a blood droplet dispensed directly on top.](image)
Chapter 2

IN-LIQUID QUALITY FACTOR IMPROVEMENT FOR FILM BULK ACOUSTIC RESONATORS BY INTEGRATION OF MICROFLUIDIC CHANNELS

2.1 Device design

We designed an FBAR device in which the liquid is confined in an integrated microfluidic channel. This structure allows a significant improvement in $Q$ over typical L-FBARs operating in liquid environments without compromising the sensitivity to mass loading, as is commonly found in shear FBARs. The approach is to form a thin liquid layer directly on top of the solid resonator, which shortens the distance the acoustic wave travels in the liquid, and thus minimizes the acoustic energy dissipation. A three-layer FBAR (Al/ZnO/Au) was fabricated on a suspended silicon nitride (SiN) membrane, and a parylene enclosure on top of the FBAR formed the microfluidic channel (Xu et al, 2009), as shown in figure 2.1(a). The channel thickness was defined by the thickness of a sacrificial layer, and inlet/outlet ports were accessed from the backside of the die.

Another design of implementing a polydimethylsiloxane (PDMS) groove ceiling with a flat glass lid to form the microfluidic channel to replace the parylene channel was also developed (Xu et al, 2011), as shown in figure 2.1(b). The major driven force for this migration was that we can take advantage of the superior acoustic properties of glass, and we could precisely control the channel thickness. The liquid thickness over the effective area of the FBAR is the vertical
distance from the top surface of the FBAR to the glass ceiling, which equals to the thickness of PDMS subtracting the thickness of FBAR.

Figure 2.1 Schematic structures for two designs of L-FBARs integrated with microfluidic channels. (a) Microfluidic channel is defined by photoresist sacrificial layer and formed by a parylene enclosure. (b) The photo-definable PDMS is patterned to a groove structure which exposes the effective area of the FBAR, and defines the channel thickness. A flat glass lid on top of the PDMS groove seals the microfluidic channel.
2.2 Modeling

To theoretically study the L-FBAR with integrated microfluidics, we used a transmission-line model to simulate this resonating system. As shown in figure 2.2, this model contains six physical layers of the L-FBAR-microfluidic channel composite, i.e. the SiN supporting membrane, Au bottom electrode, piezoelectric ZnO layer, Au top electrode, contacting liquid layer and ceiling glass lid are modeled by t-line segments of characteristic impedance \((c_{33}\rho)^{1/2}\), phase velocity \((c_{33}\rho)^{1/2}\), and length \(t\), where \(c_{33}\), \(\rho\), and \(t\) were the axial stiffness constant, mass density, and thickness of the corresponding layer, respectively. Material parameters used in the transmission-line model are listed in Table 2.1. The attenuations were only defined in the water, glass, and SiN layers. The water layer is lossy for the viscous damping. The glass layer was directly exposed to squeeze
damping and prone to a significantly larger intensity loss than other thin films of solid composites. The losses in the ZnO and metal layers were relatively small but have to be corrected to avoid an infinite $Q$ of the FBAR itself. We included the effects of Al/ZnO/Au of the FBAR into the attenuation of SiN layer. The corrected attenuation coefficient of SiN returns a $Q$ of 480 of the FBAR itself (in the air) to agree with the experimental data.

Table 2.1 Material parameters in the transmission-line model. The density and sound velocity are referred from www.memsnet.org/material/, and the attenuation coefficients of water and glass are from the references (Akashi et al, 2000) and (So 1998), respectively.

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<td>4.5×10$^{-15}$</td>
</tr>
<tr>
<td>Al</td>
<td>2700</td>
<td>6420</td>
<td>1.5×10$^{-7}$</td>
<td>-</td>
</tr>
<tr>
<td>ZnO</td>
<td>5600</td>
<td>6400</td>
<td>2.1×10$^{-6}$</td>
<td>-</td>
</tr>
<tr>
<td>Au</td>
<td>19280</td>
<td>3240</td>
<td>1.5×10$^{-7}$</td>
<td>-</td>
</tr>
<tr>
<td>Water</td>
<td>1000</td>
<td>1519</td>
<td>various</td>
<td>2.23×10$^{-14}$</td>
</tr>
<tr>
<td>Glass</td>
<td>2600</td>
<td>5500</td>
<td>5.0×10$^{-4}$</td>
<td>5.11×10$^{-13}$</td>
</tr>
</tbody>
</table>

This model was simulated using the Advanced Design System (ADS), Agilent, in an under-coupled, two-port configuration. $Q$ is defined as the inverse of the fractional bandwidth. Figure 2.3(a) showed the simulated values of $Q$ versus the thickness of the water layer, $t_{\text{water}}$, normalized to the acoustic wavelength in water, $\lambda_{\text{water}}$ which is calculated by:

$$\lambda_{\text{water}} = \frac{c_{\text{water}}}{f_r}$$  \hspace{1cm} (Eq. 2.1)

where $c_{\text{water}}$ is the acoustic velocity in water and $f_r$ is the resonant frequency. $\lambda_{\text{water}}$
is approximately 1.42 μm at 1.041 GHz which is obtained from our consequent experimental verifications for the oscillatory $Q_s$. At the very right region of this figure, $t_{\text{water}}/\lambda_{\text{water}} \gg 1$, and $Q$ approaches approximately 20, representing the case of which the resonator is fully immersed in liquid or contacting with a semi-infinitely thick water layer. At the left region of figure 2.3(a), $t_{\text{water}}/\lambda_{\text{water}} < 40$, an oscillatory pattern was observed as the water thickness continuously changed. The minimum $Q$ locates at zero water thickness, in which the thick glass lid directly sits on the top electrode surface of the FBAR. The value of $Q$ at zero water thickness is even lower than the one in the fully immersed case because the impedance mismatch between the solid FBAR body ($Z_{\text{gold}} = 63.8 \text{ MPa} \cdot \text{s/m}$) and the thick glass lid ($Z_{\text{glass}} = 12.1 \text{ MPa} \cdot \text{s/m}$) is less than the impedance mismatch between the FBAR body ($Z_{\text{gold}} = 63.8 \text{ MPa} \cdot \text{s/m}$) and contacting water ($Z_{\text{water}} = 1.483 \text{ MPa} \cdot \text{s/m}$). The glass lid is considerably thicker (0.5 mm) than other thin film layers, which leads to a significantly higher leakage (loss) compared to other layers. The minimum $Q$ values repeatedly occurred when the channel thickness was an integer multiple of a half wavelength in water ($n\lambda_{\text{water}}/2$). As the channel thickness increased, the acoustic attenuation in water was amplified exponentially; thereafter the acoustic intensity reflected from the water/glass lid interface reduced, this intensity attenuation caused a gradual reduction in the difference between the maximum and minimum values of $Q$. This damped oscillatory behavior could be also explained from the Smith chart of the system, shown in figure 2.4. The spiral represented the typical trajectory of $Z_{\text{in}}$ at the FBAR/water interface as the channel thickness increases from zero to infinity. $Q$
found its maximums when the channel thickness was an odd integer multiple of a quarter wavelength in water \(((2n-1)\lambda_{\text{water}}/4)\), where the impedance transformation through the water layer causes a large mismatch between \(Z_{Au}\) and \(Z_{in}\). \(Q\) has minimal values when the channel thickness was an integer multiple of a half wavelength \((\lambda_{\text{water}}/2)\), in which the impedance mismatch between \(Z_{Au}\) and \(Z_{in}\) were minimums.

Figure 2.3.(a) Theoretically predicted \(Q\) pattern versus the ratio of the fluidic channel thickness to the acoustic wavelength \(\lambda_{\text{water}}\); (b) measured values of \(Q\) in a magnified area of the simulation pattern at a low ratio regime.
Figure 2.4 Schematic Smith chart of the incident impedance at FBAR/water interface, $Z_{in}$. Impedances are normalized to $Z_{water}$. The spiral represented $Z_{in}$ as the channel thickness increases from zero to infinity. Maximums of mismatch between $Z_{Au}$ and $Z_{in}$ happen when the channel thickness is an odd integer multiple of a quarter wavelength ($\frac{(2n-1)\lambda_{water}}{4}$), leading high reflections of the acoustic intensity and $Q$ found its maximums. Minimums of impedance mismatch between $Z_{Au}$ and $Z_{in}$ results low reflection and minimum $Q$s at the channel thickness of an integer multiple of a half wavelength ($n\lambda_{water}/2$).

This oscillatory behavior of $Q$ could also be physically explained by the constructive or destructive interference between the reflections from FBAR/liquid and liquid/glass interfaces, the constructive interference of the two reflection components causes a good mirroring effect and high $Q$, while destructive interference leads to cancellation of the reflection and high leakage, hence lower $Q$.  

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2.3 Device fabrication

As illustrated in figure 2.5, the L-FBARs having an integrated parylene microfluidic channel were fabricated using the standard silicon micromachining technology. A 2000-Å-thick thermal oxide layer and a 4000-Å-thick low-stress SiN layer were first grown and deposited on a silicon wafer to form the supporting membrane, followed by sputtering a 1500 Å Al film, which formed the bottom electrode. The bottom electrode was patterned and etched to circular shapes (with a diameter of 100 µm) which determined the effective area of the FBARs. ZnO films with various thicknesses (0.55 µm, 2 µm, or 4 µm) were sputtered on separate wafers in a reactive chamber, and a 1500 Å Cr/Au layer was thermally evaporated to form the top electrode.

A-174 Silane was used as an adhesion promoter of parylene to treat the substrate surface. 0.5% of A-174 Silane was mixed with 49.5% of water and 50% of Isopropyl Alcohol (IPA) and was left for 2 hr before use. The wafer was soaked in the mixture for 2 hr and then air-dried for 30 min. The wafer was soaked in IPA for 5 min to remove excessive residues and was dried with N₂ stream. Note that the adhesion promoting process was critical that without it, the parylene film had very poor adhesion to the substrate, and this process had to be completed prior to the patterning process of the sacrificial photoresist layer since photoresist was soluble in IPA.

A 3 µm thick photoresist was then spun and patterned on the FBAR as a sacrificial layer that defined the fluidic channel, which was 80 µm × 12 mm × 3
µm, corresponding width, length, and thickness, respectively. A 3.5 µm thick parylene-C film was deposited on top of the sacrificial photoresist pattern to form the channel enclosure. After patterning the parylene using O₂ plasma to expose the FBAR electrodes, the wafer was etched from the backside by deep reactive ion etching (D-RIE) to form the channel inlets/outlets and to release the SiN/SiO₂ membrane. The membrane size was determined by the diameter of these backside holes, which were typically 400 µm. This is substantially larger than the effective area of FBARs so that the anchor loss could be minimized. The wafer was then soaked in acetone for overnight to remove the photoresist sacrificial layer, and was lastly transferred and dried in a supercritical CO₂ chamber to take out acetone and to release the microfluidic channels. Supercritical CO₂ dry was essential to the fabrication process as it alleviated the release-stiction problem and avoided any possible collapse of the microfluidic channels. A fabricated device is shown in Figure 2.7(a).

Figure 2.5 Fabrication process of the L-FBAR integrated with a parylene microfluidic channel.
As shown in figure 2.7 (a) and (b), the fabrication of FBARs integrated with PDMS channels inherited the same processes as the parylene ones prior to the formation of the PDMS/glass microfluidic channels. After the formation of FBARs on the substrate, we spin-coated photo-patternable PDMS (WL-5150, Dow Corning) and patterned it using UV photolithography to form the grooves (80 μm × 12 mm) of the microfluidic channels on top of the FBARs and to expose the electric connections. Consequently we sealed the channels using a glass lid to enclose the microfluidic channels. The inlet/outlet ports could be accessed either from the backside (through holes etched by DRIE) or topside of the chip (drilled holes on the glass lids). Various channel thicknesses were obtained on the same chip by repeatedly etching the unmasked PDMS groove by CH₄/O₂ reactive ion etch (RIE). A fabricated FBAR integrated with the PDMS/glass based microfluidic channel is shown in figure 2.7(b) and the cross-sectional view is shown in a scanning electron microscope (SEM) image of figure 2.8.
Figure 2.6 Fabrication process of the L-FBAR integrated with PDMS/glass microfluidic channels.
Figure 2.7 Top view of FBARs integrated with (a) parylene (b) PDMS/glass microfluidic channels, under optical microscopes.
2.8 The cross-sectional view of an FBAR with a PDMS microfluidic channel. The device was cleaved using a focused ion beam (FIB) for a width of 10 µm on the membrane so that the cross-sectional picture could be taken from the side using an SEM. Both FIB and SEM were combined in FIB/SEM Nova 200 NanoLab UHR FEG (FEI).

2.4 Experimental characterization

We first examined the $Q$ values of the parylene microfluidic channel based L-FBARs under three different circumstances: i) in air, ii) in contact with water of large depth, and iii) in water confined to the integrated microfluidic channel. These situations respectively corresponded to air damping, full damping, and partial damping to the mechanical vibrations of the resonator. The devices were characterized using the $S$ parameter, $S_{11}$, measured by an HP 8510C network analyzer. The $Q$ of the series resonance was calculated from $Q_s = \left. \frac{f_s}{2} \frac{d\phi}{df} \right|_{f=f_s}$.
where \( \phi_z \) is the phase of the impedance, \( Z \), which was extracted from the recorded \( S_{11} \) data.

The fundamental resonant frequency of an FBAR is roughly determined by the thickness of the composites:

\[
f_r = \frac{v}{2d}
\]  
(Eq. 2.2)

where \( v \) is the effective velocity and \( d \) is the total thickness of the FBAR composites. When operating in air, the fundamental resonant frequencies of the 0.55 \( \mu \)m, 2.0 \( \mu \)m, and 4.0 \( \mu \)m thick ZnO FBAR were approximately 2.0 GHz, 1.05 GHz, and 600 MHz, respectively. The impedance spectrum of the FBAR with the 2.0 \( \mu \)m ZnO layer is shown in figure 2.9(a) and zoomed in with Figure 2.9(b). The sharp resonance peak corresponded to a \( Q \) of approximately 250 in air, and the 0.55 \( \mu \)m and 4.0 \( \mu \)m-thick ZnO FBARs also possessed high \( Q \) values. The high \( Q \) obtained in air implies a small energy loss per resonance cycle. The backside hole was then filled with a water droplet to measure the \( Q \) at full damping. The depth of the water was more than 450 \( \mu \)m, which is much larger than the acoustic wavelength in water, \( \sim 1.41 \mu \)m at 1.05 GHz, calculated from (Eq. 2.1), so it could be considered practically infinite or semi-infinite depth. This mode of operation corresponded to “full damping”. A significant drop in \( Q \) to approximately 10 was observed immediately for all of the three FBAR devices. The low values of \( Q \) are resulted from the loss due to the smaller acoustic impedance mismatch at the FBAR/water interface (\( Z_{\text{gold}} = 63.8 \text{ MPa}\cdot\text{s/m}, Z_{\text{water}} = 1.483 \text{ MPa}\cdot\text{s/m} \)) than that at the FBAR/air interface (\( Z_{\text{gold}} = 63.8 \text{ MPa}\cdot\text{s/m}, Z_{\text{air}} = 0 \)). A large portion of the
acoustic intensity transmits into the water and is attenuated due to the viscous damping which scales with the square of the frequency (the attenuation coefficient is a material property and is defined as $\alpha/f^2$, see Table 2.1) and is considerably high in the GHz frequency range.

Figure 2.9 (a) Impedance spectra of a 2-μm-thick ZnO FBAR from 500 MHz to 1.50 GHz, (b) zoom-in view over the frequency range of 1.02–1.08 GHz. The impedance minimum defines the series resonance and the impedance peak corresponds to the parallel resonance.
Note that $Q$ at partial damping shows large device-to-device variation; this phenomenon may be due to process variations of the channel thickness and parylene films, as the $Q$ is very sensitive to the channel thickness which is discussed in Section 2.2. The contact liquid damping also influenced the resonant frequency of the FBAR. The resonant frequency shifted from 1.0472 GHz in air to 1.0286 GHz in semi-infinite depth water (full damping) corresponding to an effective mass loading of 29 µg/cm$^2$, which was calculated from the mass sensitivity to be discussed in Chapter 3. Phase spectra of a resonator with a 2 µm ZnO layer at different damping conditions were shown in Figure 2.10. The series resonance was defined as the frequency at which the impedance was at a minimum. The largest phase slope at resonance indicated $Q \sim 240$ in air, the smallest phase slope indicated $Q \sim 7$ at full damping, and the relatively sharp slope corresponded to $Q \sim 120$ at partial damping. The $Q$ improvement at partial damping was attributed to the integrated microfluidic channel since the acoustic wave is confined in the thin water layer. The confinement of the liquid in a microfluidic channel dramatically shortened the distance that the acoustic wave travels in the lossy liquid, and allowed a larger proportion of the acoustic energy to be reflected back from the water/enclosure interface.
Figure 2.10 The phase spectra of the L-FBAR in the air (air damping), water (full damping), and with the microfluidic channel (partial damping) near the resonant frequency. The slope of the phase indicates $Q$.

Table 2.2
Measured $Q$ for different damping conditions.

<table>
<thead>
<tr>
<th>ZnO thickness ($\mu$m)</th>
<th>0.55</th>
<th>2.0</th>
<th>4.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resonant freq. (GHz)</td>
<td>2.20</td>
<td>1.05</td>
<td>0.60</td>
</tr>
<tr>
<td>$Q$ at air damping</td>
<td>220</td>
<td>240</td>
<td>430</td>
</tr>
<tr>
<td>$Q$ at full damping</td>
<td>12</td>
<td>7</td>
<td>15</td>
</tr>
<tr>
<td>$Q$ at partial damping</td>
<td>Average value</td>
<td>38</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>Stan. dev.</td>
<td>3.5</td>
<td>25</td>
</tr>
</tbody>
</table>

To further improve the $Q$ and to better understand the $Q$ behavior in a partial damping condition, we tested the L-FBARs integrated with PDMS/glass microfluidic channels having various channel thickness. The L-FBAR consisted of a 2 $\mu$m-ZnO thin film and a resonance frequency of
approximately 1.041 GHz was measured. $Q$ was 480 while operating in the air. $Q$ dropped to approximately 10 when a macro-size water droplet was dispensed on the top surface. The diameter of the water volume was more than 450 μm, significantly larger than the acoustic wavelength in water, thus, the thickness of water was practically infinite for the resonator, which corresponded to the positions shown at the far right in figure 2.3(a). Partial damping condition was realized by FBARs integrated with PDMS/glass microfluidic channels. Six different channel thicknesses, from 4.77 μm to 5.68 μm, were experimentally obtained by repeatedly etching the unmasked PDMS groove on a same device. A glass lid sealed the channel after each etching process. $Q$ of each point was measured. The measured $Q$s were plotted on top of the simulated curve in figure 2.3(a) (a zoom-in view of figure 2.3(a) at its lower ratio region). The experimental values ranged from 50 to 150, which were within 15% of the predicted values. The value of $Q$ periodically increased and decreased as the channel thickness varied. Maximum $Q$s were found at channel thickness of 4.77 μm and 5.58 μm, close to 13/4 and 15/4 of the wavelength in water, agreed with our analytical results. The discrepancy of $Q$ values between the simulation curve and measurement points is primarily contributed by the anchor loss induced by the supporting membrane (Park and Park 2004), which was not included in the transmission line models. The experimentally obtained maximum $Q$ of 150 achieved by the PDMS/glass microfluidic at an optimized channel thickness demonstrates at least 10× to the traditional L-FBARs in liquid environments.
Chapter 3

IMPLEMENTATION OF AN OSCILLATOR USING A HIGH QUALITY FACTOR FILM BULK ACOUSTIC RESONATOR IN LIQUID FOR BIOSENSING APPLICATIONS

3.1 Sensor characterization

We characterize the sensitivity (Hz·cm$^2$/ng) and the minimum detectable mass (ng/cm$^2$) of the FBAR sensor based upon the operating frequency of 1.041 GHz and $Q$ of 150. The mass sensitivity, $s_m$, was calculated to be 638 Hz·cm$^2$/ng, by (Zhang et al, 2005):

$$s_m = \frac{f_r}{\sum \rho_i t_i}$$  \hspace{1cm} (Eq. 3.1)

where $\rho_i$ and $t_i$ denoted the density and thickness of the $i$-th layer (SiN, Al, ZnO, and Au) in the composite resonator, respectively. The minimum detectable mass was derived from the minimum detectable frequency shift ($\Delta f_{\text{min}}$), by (Rodriguez-Pardo et al, 2004):

$$\Delta m_{\text{min}} = \frac{\Delta f_{\text{min}}}{s_m}$$  \hspace{1cm} (Eq. 3.2)

To determine the minimum detectable frequency shift ($\Delta f_{\text{min}}$), we measured the short term stability of the fabricated device. Sampling time of 25s, 100 samples of the resonant frequency measurement for the FBAR was plotted in figure 3.1(a) and the Allan variance ($\sigma^2$), calculated by (Rodriguez-Pardo et al, 2004):
\[
\sigma_y^2(m, \tau) = \frac{1}{2(m-1)} \sum_{k=1}^{m-1} (y_{k+1} - y_k)^2
\]  
(Eq. 3.3)

was plotted over time in figure 3.1(b), in which \( m \) is the sampling number and \( y \) is the measurand. \( \Delta f_{\text{min}} \) of 860 Hz was then calculated from (Rodriguez-Pardo et al, 2004):

\[
\Delta f_{\text{min}}(\tau) = \sigma(\tau) \cdot f_0
\]  
(Eq. 3.4)

and suggested the FBAR contacting with water inside the microfluidic channel had a minimum detectable mass of 1.35 ng/cm\(^2\), which showed an improvement of at least 6 times over the state-of-the-art FBAR sensors in liquids (Wingqvist et al, 2007; Weber et al, 2006).

### 3.2 FBAR oscillator

The MEMS FBAR needs to be interfaced with circuitry, oscillator, on a small form factor so that the entire system can be portable and field deployable. To assemble FBAR in an oscillator, we mounted a fabricated FBAR with the optimized microfluidic channel thickness on a printed circuit board (PCB) and wire-bonded to the circuitry on the board. FBAR functions as an inductor in the oscillator circuit as shown in figure 3.2 (a).
Figure 3.1 (a) Resonant frequency sampling (sampling time: 25s, 100 samples) and (b) Allan variance on the time domain for the FBAR.
The BJT transistor (NE68119) is biased by $R_1$, $R_2$, $R_3$ and $C_3$. Effective inductance of FBAR, $C_1$ and $C_2$ determines the oscillation frequency. The effective inductance of FBAR is a function of resonant frequency of FBAR. Thus the oscillation frequency of FBAR oscillator reflects the frequency characteristics of FBAR. We fabricated the PCB using a milling machine (LPKF protomat C30s) to pattern the plated copper on both sides. A BJT transistor was located at the center of the board. Standard SMD components were soldered connecting on the board. A 50-$\Omega$ connector connected with the oscillator output to a spectrum analyzer via a standard 50-$\Omega$ cable. Two wires were soldered on the pads to connect the board to a DC power supply. The assembled FBAR oscillator is shown in figure 3.2 (b).

3.3 Biosensing

The phenomena and characterization of protein adsorption at solid/solution interface is essential for developing biocompatible materials, various biotechnological processes, and diagnostic techniques (Choi et al, 2008). In particular, blood protein adsorption on medical devices and their subsequent displacement results in false errors in diagnostics (Masson et al, 2006). Three of the most abundant blood proteins, albumin (Alb), immunoglobulin G (IgG), and fibrinogen (Fib), are most expected to be in a protein layer adsorbed to the surface of medical devices in contact with blood, since they play a critical role on medical devices as their subsequent displacement may cause false errors in diagnostics (Ostuni et al, 1999).
Figure 3.2 (a) Schematic circuitry of the FBAR oscillator and (b) a photograph of an assembled FBAR oscillator in operational status.
Competitive protein adsorptions from complex solutions are involved with sequential protein adsorption and displacement of adsorbed proteins on the surfaces. These processes are described by the Vroman effect, also known as Vroman cascade, which is an important phenomenon describing low-affinity proteins pre-adsorbed on a surface are displaced by high-affinity proteins (Vroman and Adams 1969). It is a competitive adsorption/exchange of proteins behaviour, for which higher mobility proteins (low-molecular weight (LMW) proteins) arrive first and are later replaced by less motile proteins (high-molecular weight (HMW) proteins) that have a higher affinity for the surface. The reverse sequence does not occur: as HMW proteins cover the surface first, LMW ones arriving later do not replace the former proteins (Choi and Chae 2009; Wertz and Santore 2001; Schmaier et al, 1984). Using the MEMS FBAR, we could monitor the competitive adsorption of proteins in real-time which facilitates the understanding of protein adsorption at solid/solution interface.

We used the L-FBAR as a mass sensor for monitoring the competitive adsorption of proteins (Xu et al, 2011). The sensor was featured with a 1.0 μm-thick ZnO thin film and had a resonant frequency of approximate 1.55 GHz. The thickness of the integrated microfluidic channel was optimized to 3.9 μm, close to $17/4\lambda_{\text{liquid}}$. The FBAR sensor exhibited a high $Q$ of 130 in liquid and the mass sensitivity, $s_m$, of the FBAR sensor was calculated to be 1358 Hz·cm$^2$/ng.

We monitored Alb, IgG, and Fib as protein layers adsorbed to the
sensor surface. The LMW protein, Alb (M$_w$ of 67 kDa), was designed to initially cover the sensor surface, and then being replaced by higher molecular weight proteins, IgG and Fib. A reverse injection sequence was also conducted. We initially let Phosphate Buffered Saline (PBS) flow in a constant rate of 1 μL/min in the microfluidic channel by two external syringe pumps (PHD 22/2000 Syringe Pump from Harvard Apparatus). We recorded the resonant frequency of the FBAR as the frequency baseline.

We first injected Alb (0.1% w/v in PBS) solution for 90 seconds and switched back to PBS flow. After a complete washing of the sensing surface by the buffer solution, we replaced the Alb with IgG (0.1% w/v in PBS) for another 90 seconds, and then switched to PBS again. This process repeated itself for the Fib (0.1% w/v in PBS) injection followed by buffer washing. The resonant frequency of the FBAR was measured by a network analyzer (Agilent 5071C) and the output data was acquired via LabVIEW. The flow of liquid solution in the microfluidic channel was controlled at a constant rate of 1 μL/min. The reverse sequence protein adsorption was conducted in a similar manner: after forming the baseline by flowing PBS, Fib, IgG, and Alb with same concentration (0.1% w/v in PBS) were sequentially injected to the microfluidic channel.

As shown in figure 3.3 (a), The FBAR had resonant frequencies of 1.5461GHz and 1.5457 GHz before and after the sensor surface was adsorbed with lowest molecular weight protein, Alb; both measured in PBS. This frequency shift, -400 kHz corresponded to a mass density change of 295
ng/cm², comparing to 260-500 ng/cm² or 2.6-5.0 mg/m² reported in (McClellan and Franses, 2005). After the ejection of the higher molecular weight protein, IgG (Mₙ of 150 kDa), the resonant frequency changed to 1.5450 GHz indicating a frequency downward shift of 700 kHz due to the displacement of Alb on the sensor surface by IgG. The frequency shift of -1100 kHz from the original state (before Alb adsorption) relates to a mass density change of 810 ng/cm² or $3.25 \times 10^{12}$ molecules/cm² if converted to the surface coverage density of IgG, which is higher than the theoretical limit of densely packed IgG in a monolayer, 2.1 pmol/cm² (Liao et al, 2004) or 1.26 $\times$ $10^{12}$ molecules/cm², hence suggesting the IgG aggregation of multilayer at the sensor surface. Sequentially, the highest molecular weight protein, Fib (Mₙ of 340 kDa), displaced IgG showing a resonant frequency shift of -1100 kHz from IgG surface or a frequency shift from original state of -2200 kHz, which represents a loading mass of 1620 ng/cm² or a coverage of $2.86 \times 10^{12}$ molecules/cm². Comparing to the low and high surface coverages of $1.8 \times 10^{11}$ molecules/cm² and $8.9 \times 10^{11}$ molecules/cm² reported by Jiroušková et al (in 2007), this surface coverage is very dense, implying a completely interconnected fibrinogen pattern in a monolayer or even aggregated for multilayer. When we reversed the injection sequence of the proteins on another sensor which had a slightly different baseline resonant frequency (1.5906 GHz), as shown in figure 3.3 (b), the resonant frequency shifts were negligible: both of IgG and Alb injection generated the frequency shifts no more than 10 kHz. It suggested that little replacement occurred at the sensor
surface for the reverse sequential protein injections. Based upon the in-situ real time measurements data, the FBAR has an approximate 10-bit resolution using the minimum detectable frequency shift, $\Delta f_{\text{min}}$, of 860 Hz.

Figure 3.3 Measured resonant frequency response of the L-FBAR sensors for the protein characterization of Vroman effect: (a) when a LMW protein adsorbs first to the FBAR surface and HMW proteins arrive latter (Alb $\rightarrow$ IgG $\rightarrow$ Fib) and (b)
for the reversal sequence: when a HMW protein adsorbs first and LMW proteins come later (Fib→IgG→Alb).

We performed similar experiments to characterize the FBAR oscillator. The FBAR oscillator was characterized for its time domain frequency stability. Sampling time of 25s, 100 samples of the resonant frequency measurement for the FBAR oscillator is plotted in figure 3.4 (a) and the Allan variance for the oscillator is shown in figure 3.4 (b). The minimum detectable frequency shift, $\Delta f_{\text{min}}$, of 11 kHz was obtained. This relatively large minimum detectable frequency was confirmed by the measurement of the phase noise for the FBAR oscillator, as shown in figure 3.5. The power spectral density of the oscillator at 10 kHz offset was measured as only -60 dBc/Hz. Such high phase noise was mainly because the practical implementations including the bonding wire reduced the equivalent Q of the resonator due to the addition of the wire resistance.
Figure 3.4  (a) Oscillatory frequency sampling (sampling time: 25s, 100 samples)  
(b) Allan variance on the time domain for the FBAR oscillator.
Figure 3.5  Phase noise of the FBAR oscillator measured by a signal analyzer. Power spectral density of -60 dBc/Hz is measured at 10 kHz offset, indicating a high phase noise, which is most likely due to the wire resistance introduced by the wire bonding.

We used the FBAR oscillator to monitor the competitive adsorption of proteins. Same proteins and sequences as previous experiments were used. Figure 3.6 showed the oscillation frequency response according to the protein adsorptions to the sensor surface and the consequential protein exchanges. The adsorption of Alb caused an oscillation frequency shift ($\Delta f_1$) of -60 kHz, corresponding to a mass density increase of 144 ng/cm$^2$. The displacement of Alb on the sensor surface by IgG resulted a frequency downshift of 160 kHz ($\Delta f_2 - \Delta f_1$), indicating a mass density change of 383 ng/cm$^2$. Lastly, the highest
molecular weight protein, Fib, displaced IgG, causing an oscillation frequency shift of 310 kHz ($\Delta f_3 - \Delta f_2$), which represented an added mass of 743 ng/cm$^2$. The absolute frequency shift values were significantly smaller than the previously studied FBAR sensor itself because the FBAR component in the oscillator was featured with a thicker ZnO film (~1.5 μm) and thus had a lower resonance frequency and a smaller mass sensitivity. The measured mass density change was slightly smaller than the former FBAR sensor itself. This discrepancy might be due to non-perfect cleanliness of the Au sensing surface, different Au surface roughness, which resulted in different surface adsorption ratio (Veiseh et al, 2002; Höök et al, 2002).

Figure 3.6  Oscillation frequency response of the FBAR oscillator for the protein characterization of Vroman effect in a forward sequence (Alb→IgG→Fib).
Chapter 4

CONTOUR-MODE FILM BULK ACOUSTIC RESONATOR FOR
MONITORING WHOLE BLOOD COAGULATION IN REAL TIME

4. 1 Device design

L-FBARs achieve high $Q$ in liquids by integrating microfluidic channels; however, the unavoidable fact of squeeze damping and the critical height requirements of the microfluidic channel limit further improvements. Contour-mode FBARs (C-FBARs) are excited in its radial directions, in which the vibration displacement is parallel to the resonator/liquid interface. The shear viscous damping, instead of the squeeze damping in L-FBARs, alleviates the acoustic energy loss and consequently results in high $Q$s. C-FBARs show a substantially improved $Q$ in a semi-infinite depth liquid environment of which a liquid droplet is in direct contact with the top electrode and no microfluidic channel is required (Xu et al, 2010). Compared to the traditional L-FBARs, C-FBARs have lower resonant frequencies (100~200 MHz), yet they exhibit significantly higher $Q$s and, consequently, notably improved frequency resolutions. Meanwhile, C-FBARs are sensitive to the viscosity change of the contacting liquids, thus can be deployed as an effective viscosity sensing device. Based on the viscosity sensing scheme, the C-FBARs demonstrate the capability of monitoring blood coagulation continuously for blood rheology.

Figure 4.1 (a) illustrates the schematic of the MEMS C-FBAR. The C-FBAR consists of a ring-shaped piezoelectric aluminum nitride (AlN) thin film sandwiched between the top and bottom gold electrodes; the ring is suspended
and connected to the silicon substrate with one or multiple anchors. The resonator is excited in a pure radial-extensional mode. The C-FBAR operates at its fundamental resonant mode of which the frequency $f_0$ can be roughly determined by the width of the AlN ring, $W$, if any surface detail is ignored (Piazza et al, 2006):

$$f_0 \approx \frac{1}{2W} \sqrt{\frac{E_P}{\rho(1-\sigma^2)}}$$  \hspace{1cm} (Eq. 4.1)

where $\rho$ is the density, $E_p$ and $\sigma$ are the in-plane Young’s modulus and Poisson’s ratio of AlN, respectively.

The central circle left on the substrate is to avoid liquid smearing beneath the AlN ring composite. The distance between the suspended ring and the substrate/central disk is approximately 10 µm, which is significantly larger than the penetration depth (~ 88 nm in blood at room temperature) of the acoustic shear wave into the contacting liquids, thus, the crosstalk is considered negligible.

The dimensions of the MEMS sensors are listed in Table 4.1.

<table>
<thead>
<tr>
<th>AlN thickness</th>
<th>Inner diameter</th>
<th>Outer diameter</th>
<th>Anchor width</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0 µm</td>
<td>110 µm</td>
<td>150 µm</td>
<td>10 µm</td>
</tr>
</tbody>
</table>
Figure 4.1 (a) Schematic of a MEMS contour-mode film bulk acoustic resonator (C-FBAR) with a blood droplet dispensed directly on top of the C-FBAR; (b) An SEM image of a fabricated single-anchor C-FBAR. The central circle is to avoid liquid smearing beneath the AlN ring composite; (c) top-view of a multi-anchor C-FBAR under an optical microscope.
4.2 Modeling

The mass sensitivity, $s_m$, of a C-FBAR is:

$$s_{m,\text{CFBAR}} = \frac{1}{2} \sum_i f_r \rho_i t_i$$

where $\rho_i$ and $t_i$ denoted the density and thickness of the $i$-th layer (bottom electrode, AlN, and top electrode) in the composite resonator, respectively. Comparing to the mass sensitivity of a L-FBAR: $s_{m,\text{LFBAR}} = \sum_i f_r \rho_i t_i$, it is usually smaller because of the lower fundamental resonant frequency (150 MHz in C-FBARs compared to ~1 GHz in L-FBARs). However, the resonant frequency of a C-FBAR is selectively sensitive to the viscosity (shear viscosity) of the contacting liquids due to the vibration mode, (L-FBAR is sensitive to both density and viscosity) thus being chosen for a viscosity sensor.

An unperturbed (in vacuum or proximate air environments) C-FBAR can be modeled with a lumped element equivalent circuit, as shown in figure 4.2. $R_e$ is the series resistance of electrodes; $C_0$ is the static parallel capacitance of the device; $R_1$, $L_1$, and $C_1$ are, in accordance with the Butterworth Van Dyke (BVD) model, the motional components of the resonator, resistance, inductance, and capacitance, respectively (Piazza et al, 2006).

$$C_0 = \varepsilon_0 \varepsilon_{33} \frac{2\pi r_{ave} w}{t}$$
$$R_1 = \frac{\pi^2}{8} \frac{t}{2\pi r_{ave}} \frac{\rho^{3/2}}{E_p^{3/2} Q d_{31}^2}$$
$$C_1 = \frac{8}{\pi} \frac{2wr_{ave}}{t} d_{31}^2 E_p$$
$$L_1 = \rho \frac{wt}{8} \frac{1}{2\pi r_{ave} d_{31}^2 E_p^2}$$

(Eq. 4.2)

where $\varepsilon_{33}$ is the relative dielectric constant of AlN in the out-of-plane direction, $t$
is the thickness, $\rho$ is the density of the AlN film, $w$ is the ring width, $r_{ave}$ is the average radius of the ring, $d_{31}$ is the piezoelectric coefficient, and $E_P$ is the equivalent Young’s modulus of AlN, respectively. A perturbation caused by a fluid contact on the top surface is described by two additional elements: a motional inductance $L_2$ and resistance $R_2$ (Martin et al, 1993; Ballantine et al, 1997):

$$L_2 = \frac{L_1}{\pi} \left(\frac{4\pi f \rho_w \eta_w}{\mu\rho}\right)^{1/2} \quad R_2 = 2\pi f L_2$$  \hspace{1cm} (Eq. 4.3)

where $f$ is the frequency, $\mu$ is the shear stiffness of AlN, $\rho_w$ and $\eta_w$ are the liquid density and viscosity, respectively.

Figure 4.2 Lumped-element BVD (Butterworth Van Dyke) models for (a) an unperturbed C-FBAR in the air; (b) a perturbed C-FBAR in contact with liquid.
The $Q$ and series resonant frequency of the C-FBAR can be determined by the $LCR$ parameters as:

\[ Q_{air} = \frac{2\pi f L_1}{R_e + R_1} \]  
\[ (Eq. 4.4) \]

\[ Q_{liquid} = \frac{2\pi f (L_1 + L_2)}{R_e + R_1 + R_2} \]  
\[ (Eq. 4.5) \]

\[ f_{liquid} = \frac{1}{2\pi \sqrt{(L_1 + L_2)C_1}} \]  
\[ (Eq. 4.6) \]

in which $L_2$ is a function of the viscosity at a given liquid density (Eq. 4.3). Therefore, viscosity change induces resonant frequency change and by monitoring the resonant frequency change in time domain we can correlate the frequency shift to the viscosity change of the target media, inversely.

Be noted, a L-FBAR can also be effected by the viscosity change of the coupled liquid, but possessing a lower sensitivity since the mechanical vibration is perpendicular to the sensor-liquid interface. The viscosity influenced the acoustic attenuation coefficient, $\alpha$, by Stokes equation (Dukhin and Goetz, 2009):

\[ \alpha = (2\pi^2 f^2 / \rho C^3) \left\{ \left[ (\gamma - 1)/C_\rho \right] \kappa + \frac{3}{4} \eta_s + \eta_v \right\} \]  
\[ (Eq. 4.8) \]

where $C$ is the velocity of sound; $\rho$ is the density; $\eta$ is the viscosity (shear and volume) $C_\rho$ is the specific heat at constant pressure; $\kappa$ is the thermal conductivity and $\gamma$ is the ratio of specific heats; and the acoustic attenuation coefficient is directly linked to the resonant frequency. Unfortunately, because of the complexity of the L-FBAR and integrated microfluidic channels, the analytical expression of the viscosity sensitivity become difficult, but a numerical method
could be alternatively used based upon the transmission-line model (discussed in Chapter 2) to determine the resonant frequency-viscosity relationship.

Suppose we have three common liquids: water, acetone (less viscous and lower density, comparing to water), and blood (more viscous and slightly higher density than that of water), as shown in Table 4.2 (a) and (b). If we compare water and acetone, the effective mass effect (assuming the two liquids having the same viscosity but different density) shifts the resonant frequency of a L-FBAR by 898 ppm, and the viscosity effect (assuming them having the same density but different viscosity) shifts the frequency by 108 ppm. If we compare water to blood, the effective mass effect results a resonant frequency shift of -186 ppm while viscosity effect results -607 ppm. Here the L-FBAR is assumed to have a resonant frequency of approximately 1.3 GHz and being integrated with an optimized microfluidic channel with the thickness of 3 µm (close to 2.75λ). It is clear that the L-FBAR is more sensitive to mass rather than viscosity. On the other hand, C-FBARs show the opposite trend. As shown in Table 4.2 (c) and (d), if we compare water, acetone, and blood, the mass effect can only result frequency shifts of 35 ppm and -5 ppm, respectively, but the viscosity effect allows the resonant frequency to shift 118 ppm and -308 ppm, respectively. Therefore, we conclude that although L-FBARs have slightly higher sensitivity to viscosity than C-FBARs, the selective frequency response to viscosity, rather than to the effective mass, makes the C-FBAR a better candidate for viscosity sensor than the L-FBAR.
Table 4.2
The effective mass and viscosity effects of the coupled liquids to the L-FBAR and C-FBAR sensors: (a) Viscosity effect to the L-FBAR sensor (assuming the liquids have the same density of water).

<table>
<thead>
<tr>
<th>Liquid</th>
<th>Water</th>
<th>Acetone</th>
<th>Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viscosity (cP)</td>
<td>0.894</td>
<td>0.306</td>
<td>4</td>
</tr>
<tr>
<td>$f$ (GHz)</td>
<td>1.312661</td>
<td>1.312803</td>
<td>1.311346</td>
</tr>
<tr>
<td>$\Delta f$ (ppm)</td>
<td>--</td>
<td>108</td>
<td>-607</td>
</tr>
</tbody>
</table>

(b) Effective mass effect to the L-FBAR sensor due to the different density of the liquids (assuming the liquids have the same viscosity of water).

<table>
<thead>
<tr>
<th>Liquid</th>
<th>Water</th>
<th>Acetone</th>
<th>Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density (kg/cm$^3$)</td>
<td>$1.0 \times 10^3$</td>
<td>$0.78 \times 10^3$</td>
<td>$1.05 \times 10^3$</td>
</tr>
<tr>
<td>$f$ (GHz)</td>
<td>1.312661</td>
<td>1.311484</td>
<td>1.312905</td>
</tr>
<tr>
<td>$\Delta f$ (ppm)</td>
<td>--</td>
<td>898</td>
<td>-186</td>
</tr>
</tbody>
</table>

(c) Viscosity effect to the C-FBAR sensor (assuming the liquids have the same density of water).

<table>
<thead>
<tr>
<th>Liquid</th>
<th>Water</th>
<th>Acetone</th>
<th>Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viscosity (cP)</td>
<td>0.894</td>
<td>0.306</td>
<td>4</td>
</tr>
<tr>
<td>$f$ (MHz)</td>
<td>217.579</td>
<td>217.605</td>
<td>217.512</td>
</tr>
<tr>
<td>$\Delta f$ (ppm)</td>
<td>--</td>
<td>118</td>
<td>-308</td>
</tr>
</tbody>
</table>

(d) Effective mass effect to the C-FBAR sensor due to the different density of the liquids (assuming the liquids have the same viscosity of water).

<table>
<thead>
<tr>
<th>Liquid</th>
<th>Water</th>
<th>Acetone</th>
<th>Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density (kg/cm$^3$)</td>
<td>$1.0 \times 10^3$</td>
<td>$0.78 \times 10^3$</td>
<td>$1.05 \times 10^3$</td>
</tr>
<tr>
<td>$f$ (MHz)</td>
<td>217.579</td>
<td>217.586</td>
<td>217.578</td>
</tr>
<tr>
<td>$\Delta f$ (ppm)</td>
<td>--</td>
<td>35</td>
<td>-5</td>
</tr>
</tbody>
</table>

4.3 Fabrication

The C-FBAR was fabricated with a two-mask process using the standard silicon micromachining technology, as shown in figure 4.3. Initially deposited 0.6 µm-thick low-stress SiN acted as an isolation layer, followed by the 100 nm-thick
Cr/Au bottom electrode deposition and patterning. 1.0 μm-thick piezoelectric AlN was sputtered using a single-module AMS physical vapor deposition (PVD) sputtering tool (Tegal Corp., USA). Top electrode was formed by depositing and patterning another 100 nm-thick Cr/Au film. AlN was consequently dry-etched by a Cl\textsubscript{2} based reactive-ion etching (RIE) (Ar: 10 sccm Cl\textsubscript{2}: 10 sccm, BCl\textsubscript{3}: 10 sccm, chamber pressure: 50 mTorr) to shape the suspended resonator at 200 W for 12 mins. The SiN layer was continuously dry-etched to expose the silicon substrate. The ring-shaped resonator was finally released from the silicon substrate by dry-etching the silicon using XeF\textsubscript{2} vapor (XACTIX Inc.) at 2.7 Torr for 100 cycles of 90 sec each.

Figure 4.3 The fabrication process of the MEMS C-FBAR: (a) low-stress SiN was deposited as an isolation layer, followed by Cr/Au bottom electrodes formation; (b) AlN was sputtered and top electrodes (Cr/Au) were formed; (c) Top electrodes (Cr/Au) were wet etched; (d) AlN and SiN layers were dry etched to form the resonator and to expose the Si substrate; (e) The suspended ring was dry-released by XeF\textsubscript{2} and the PR mask, finally, was removed by O\textsubscript{2} ashing.
4.4 Sensor characterization

A C-FBAR (20 μm width and 90 μm inner radius ring) was experimentally characterized in the air and in water. We measured the one-port reflection coefficient ($S_{11}$) using a network analyzer (HP 8510C). The experimental $Q$ values of the series resonance were obtained from

$$Q_s = \left. \frac{f_s}{2} \frac{d\phi_z}{df} \right|_{f=f_s},$$

where $f_s$ is the frequency of the series resonance and $\phi_z$ is the phase of the impedance, $Z$, extracted from the recorded $S_{11}$ data. The theoretical and experimental values of $Q_s$ in the air and in contact of deionized water are shown in Table 4.3.

Table 4.3
Theoretical and experimentally measured $Q$ values.

<table>
<thead>
<tr>
<th></th>
<th>In the air</th>
<th>In liquid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theoretical $Q$</td>
<td>404</td>
<td>210</td>
</tr>
<tr>
<td>Measured $Q$</td>
<td>317</td>
<td>189</td>
</tr>
</tbody>
</table>

Figure 4.4 shows an impedance plot of a C-FBAR in the air and in water. Measured $Q_s$ in the air range from 250 to 310. These values were lower than previously reported $Q_s$, partially because of the relatively large resistance, $R_e$, (~10 Ω) of our long electrodes which were designed to leave room for a sample droplet. A water droplet was dispensed directly on top of the device. As the diameter of droplet was significantly larger than the acoustic decay length in water, $\delta \sim 45$ nm at 160 MHz, the depth of droplet was treated as semi-infinite. The resonant frequency shifted downwards by 310 kHz and $Q$ became 189 due to
the effects from the motional components, $L_2$ and $R_2$, respectively; yet the $Q$ of 189 demonstrates a 13~19 × improvement over the previously reported L-FBARs in liquids.

![Figure 4.4 Measured impedance plot of a fabricated C-FBAR (20 µm width and 90 µm inner radius ring-shaped) in the air and in water showing $Q$ of 317 and 189, respectively.](image)

We also characterized the C-FBAR sensor with aptamer – thrombin binding pairs. The top gold electrode of the C-FBAR was modified with COOH-terminated self-assembly monolayer (SAM), and then washed the electrode with ethanol and water. Then, a custom-designed aptamer was immobilized on top of the COOH-terminated SAM. The aptamer sequence was designed at the 5’-terminus with an amine based linker (5’-NH$_2$-(CH$_2$)$_6$-TTC CAA CGG TTG GTG TGG TTG G-3’). After washing the surface with a buffer solution (1 × Phosphate
Buffered Saline (PBS), a droplet of thrombin solution (2 µM) was dispensed to let the thrombins bind to the aptamers and the unbounded thrombins were washed away with PBS. Figure 4.5 shows the major test sequence and the resonant frequency changes. The frequency shifts were measured in the washing steps after each chemical process in order to minimize the frequency variations resulted from the device being contacted with different liquids. The aptamer – thrombin bindings results a frequency shift of 28.4 kHz which corresponds to a mass loading of 252.8 ng/cm² via a mass sensitivity of 112 Hz·cm²/ng.

![Graph showing frequency shifts](image)

Figure 4.5 Measured resonant frequency shifts of the C-FBAR sensor due to the mass loading on the top gold electrode; (i) the C-FBAR is in contact with water, (ii) COOH-terminated SAM are formed, (iii) aptamers are immobilized, and (iv) thrombins are bound to the aptamers.
4.5 Viscosity characterization

We chose aqueous glycerine solutions to characterize the C-FBAR. Glycerine is a well-characterized liquid for a large viscosity range and density at room temperature (Segur and Oberstar 1951). Different amounts of glycerine (Sigma-Aldrich inc.) were mixed with deionized water to obtain a wide range of viscosities. Glycerine percent weight ranging from 0 to 70 resulted viscosities from 1 to 22.5 cP (centipoise) at room temperature. The sensor specifications are listed in Table 4.4.

Table 4.4
C-FBAR sensor specifications

<table>
<thead>
<tr>
<th>Resonant frequency</th>
<th>Q</th>
<th>Sensitivity</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>150 MHz</td>
<td>140 (water)</td>
<td>-31 kHz/cP</td>
<td>1 ~ 22.5 cP</td>
</tr>
<tr>
<td></td>
<td>77 (blood)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 4.6 shows the experimental setup. We placed the C-FBAR on a probe station and connected it to a LabView interfaced network analyzer (Agilent 5071C) to measure the one-port reflection coefficient ($S_{11}$). C-FBARs were first tested in the air for an unperturbed condition, and consequently calibrated using the aqueous glycerine solutions with glycerine percent weights of 0, 10, 20, 30, 40, 50, 60, and 70, respectively. The small liquid droplets were dispensed on the top surface of the C-FBARs by a syringe tip controlled by a micromanipulator under an optical microscope. The micromanipulator allowed a precise control of 3-axis movements, so that the minute volumes of liquid could be dispensed. This dedicated operation could be practically replaced by simply spreading a blood
sample on the C-FBAR surface if the sensor was packaged in which only the active surface was exposed.

Figure 4.6 Experimental setups: C-FBAR sensors were placed on an air suspended probe station and connected to a LabView interfaced network analyzer using a RF probe (Cascade Microtech, Inc.).

The C-FBARs operated at the fundamental resonant frequency of 150 MHz. The resonators possessed $Q$ of 150 to 240 in the air, defined at their series resonant frequency. These values were lower than previously reported $Q$s, mainly because of the anchor loss from the multiple supporting beams. We designed the multiple beams to provide a more robust supporting structure, as shown in figure 4.1(c). When a liquid (deionized water) droplet was dispensed directly on the top surface of the device, the resonant frequency shifted downwards by approximate
310 kHz and $Q$ decreased to ~140, due to the effects from $L_2$ and $R_2$, the motional inductance, and motional resistance, respectively. The $Q$ decreases further as C-FBARs in contact with media of higher viscosity (glycerine solutions and blood in this study). $Q$ values of 57 and 77 were observed at room temperature for 70% weight aqueous glycerine solution and citrated blood sample, respectively.

The viscosity characterization of the C-FBARs is shown in figure 4.7. The theoretical values (red line) were derived from the single side perturbed model, the frequency–viscosity observed a quasi-linear relation in this viscosity regime, showing a viscosity sensitivity of approximately -31 kHz/cP. Four repeated measurements were performed on three different C-FBARs. Measured resonant frequencies (green line) agreed well with the theoretical values at the low viscosity regime (below 10 cP). The resonant frequency shifted from 149.5125 MHz to 149.1548 MHz, corresponding to the viscosity change from 1.01 to 10.80 cP. However, the measurement deviated substantially from the predicted curve, and presented a large measurement error bar for the high viscosity liquid (70% aqueous glycerine solution), showing a limitation of the C-FBAR as a viscosity monitoring unit in a high viscosity regime. This effect agrees well with the non-Newtonian fluid model. As the viscosity sensor operates at a high frequency it is necessary to account the relaxation effects in the liquid. The relaxation effects can be modeled as a Maxwellian viscoelastic fluid, of which the shear behavior becomes non-Newtonian as the liquid viscosity increases (Ricco and Martin 1987).
Figure 4.7 Viscosity characterization of the C-FBAR using controlled glycerine solutions: theoretical (red) and experimental (green) resonant frequency shifts as a function of the viscosity in centipoise (cP).

4.6 Real-time blood coagulation monitoring

Blood samples were purchased from Innovative Research, MI. The whole blood was collected from healthy donor(s) and was treated with sodium citrate as an anticoagulation agent. The blood samples were stored at 4 °C before use. Blood viscosity has a strong function of temperature, varying from approximately 3 cP (37 °C) to 7 cP (10 °C) (Zhou et al, 1999), thus, we restored the blood samples to room temperature before measurements and performed all blood coagulation tests at room temperature to avoid the temperature effect. The coagulation process was initiated in-vitro by adding calcium ions (Ca$^{2+}$). We mixed different amounts of CaCO$_3$ powder (Sigma-Aldrich inc.) with the blood
samples, gently agitated the containing tube, and transferred a droplet of 100 nano-litter to the C-FBAR surface immediately. The resonant frequency was recorded in real time after the sample droplet was dispensed via a LabVIEW program.

We dispensed a droplet of Ca$^{2+}$ spiked whole blood directly on top of the C-FBAR to monitor the viscosity change in real time. Empirically, approximately 10 mg CaCO$_3$ in 1 mL blood sample was required to initiate the coagulation cascade. We added four different amounts of CaCO$_3$ powders to the blood samples to obtain concentrations of 10, 20, 50, and 100 mg/mL. Figure 5.8 shows the real time frequency responses of the C-FBAR monitoring the blood coagulation process initiated by 10 mg/mL Ca$^{2+}$ ions. Initially, the frequency remained almost constant. During this period, the enzymatic cascade occurred: the blood sample was activated by excessive Ca$^{2+}$ ions; in the pathway of coagulation, thromboplastin activates factor VII, which leads to the activation of factor X in turn; together with activated factor V, calcium ions and factor X formed the prothombinase complex; finally, this complex catalyzes the conversion of prothrombin to thrombin (Macfarlane 1964). During this cascade, no viscosity changes occurred, resulting a plateau phase. Consequently, at the end of the coagulation cascade, thrombin as central protein cleaved fibrinogen to fibrin. The fibrin monomers eventually polymerized into fibers. As a result of the build-up and growth of polymerization clusters the viscosity of the liquid increased (Muller et al, 2010) which represented a continuous decrease of the resonant frequency. The completion of fibrin generation or polymerization
resulted in a steady state with no further change of the resonant frequency. This real-time frequency plot offers three parameters: coagulation time, clot strength, start/end of the fibrin generation/polymerization, which are commonly used in monitoring blood coagulation process (Vikinge et al, 2000; Muller et al, 2010, Guhr et al, 2005), as indicated in figure 4.8.

Higher Ca$^{2+}$ ion concentrations expedited the coagulation process and resulted higher clot strength. As shown in figure 4.9, for blood samples from donor 1, with 10 mg/mL Ca$^{2+}$ ions, the coagulation time, indicated by the end of fibrin creation process after the blood coagulation cascade, was approximate 15 min 30 sec; with higher Ca$^{2+}$ ion concentrations, the coagulation time decreased consistently. Among the four samples, highest Ca$^{2+}$ ions concentration (100 mg/mL) produced the shortest coagulation time of the blood, approximate 5 min. The resonant frequency shifts, $\Delta f$, indicate the clot strength. $\Delta f$ ranging from 900 kHz to 2 MHz were obtained from the four samples from the same donor, which suggest very large viscosity changes of the blood produced at the end of fibrin generation. The coagulation with 20 mg/mL Ca$^{2+}$ initiator observed an abnormal smaller frequency shift than the shift caused by a lower initiator concentration. It is likely due to the non-uniform distribution of CaCO$_3$ powders in the blood droplet.

Blood samples from two donors were investigated using multiple C-FBARs. Coagulation characterization of blood samples from donor 2 were also plotted in figure 4.9. The main two parameters, clot strength and coagulation time, presented individual differences between donors. As a comparison, the thickest
aqueous glycerine solution we characterized (70%) caused a frequency shift of ~960 kHz. The large frequency shifts are mainly contributed by the very high viscosity changes of the clotting blood samples which can range from ~10 cP to ~1000 cP, and are far beyond the viscosity range that the C-FBAR can provide effective measurements. However the need of monitoring viscosity of normal blood is usually less than 10 cP (Dintenfass 1964), within the C-FBAR’s measurement range. Another uncertainty of the viscosity measurement is that the blood sample can behave as a non-Newtonian fluid. Firstly, red blood cells (RBCs) which compose about 35-40% of the whole blood volume are deformable and contribute to shear rate dependence and viscoelastic behavior (Bandey et al, 2004). Secondly, for the operating frequency and targeted viscosity range, the relaxation effects become even more significant than that in the high concentration glycerine solutions (Ricco and Martin 1987).

![Figure 4.8 Real-time frequency response of the C-FBAR monitoring the coagulation process of a citrated blood with 10 mg/mL Ca^{2+} concentration.](image_url)
Figure 4.9 Coagulation time and clot strength of the \textit{in-vitro} blood coagulation resulted from different Ca$^{2+}$ concentrations. D1, D2 denote the donors of the samples.
Chapter 5

CONCLUSION

5.1 Conclusions

This dissertation presented two novel FBAR designs which possess high $Q$ performance in liquid environments and explored various bio-sensing applications.

In its simplest form, a FBAR consists of a piezoelectric thin film sandwiched between two electrodes. When an A. C. with appropriate frequencies is applied, the FBAR can be mechanically exited in longitudinal or shear mode. FBARs were commercially developed for filters in wireless communication circuits, and recently, extended their applications to label-free bio/chemical sensors based on a “mass loading-frequency shift” scheme. FBAR sensors encountered a major challenge while operating in biologically/chemically-relevant media, as the viscous damping to the compressional vibration at the FBAR/liquid interface significantly lowered the $Q$ and accordingly resulted poor sensing resolution.

We approached high $Q$ in liquid for L-FBARs by integrating microfluidic channels to them. Confining by a microfluidic channel, the liquid had a thickness comparable to the acoustic wavelength, consequently allowed the acoustic wave to be reflected to the resonator improving $Q$ by alleviating energy loss per resonance cycle. A ZnO based L-FBAR operating at 1.04 GHz presented $Q$s of 240 in the air and 7 in a water droplet without having the microfluidic channel. Substantial improvement was achieved with our microfluidic channel integration,
that $Q$ of 120 was experimentally obtained which was one order higher than that of the state-of-the-art.

Analytical modeling based on a transmission-line model indicated a damped oscillatory variation of $Q$ when the L-FBAR is coupled with a liquid layer with finite thickness. The physical explanation is that the constructive and destructive interference between the reflections from FBAR/liquid and liquid/channel interfaces alternatively happened. The constructive interference of the two reflection components enhances a mirroring effect and produces high $Q$, while destructive interference leads to cancellation of the reflection and high leakage, hence lower $Q$. We developed a L-FBAR with PDMS/glass microfluidic channel of which the channel thickness could be precisely controlled. Experimental results verified the theoretical prediction: maximum $Q$ values were periodically obtained at the channel thickness that is an odd integer multiple of the quarter wavelength, and minimum $Q$s located at even ones. By optimizing the channel thickness to $13/4\lambda$ and taking the advantage of high acoustic impedance of glass ceiling, $Q$ of 150 was observed which pointed to a sensitive, high-resolution sensors for bio-chemical analyte detection in applications involving liquid media.

The high $Q$ L-FBAR with microfluidic integration was implemented to an oscillator. The sensor was characterized and a minimum detectable frequency shift of 860 Hz was extracted from stability measurements. The corresponding mass resolution of 1.35 ng/cm$^2$ showed an improvement of 6 times over that of the current technologies. The FBAR sensor demonstrated the detection of aptamer -
thrombin pair bindings at the sensor surface, and further monitored competitive adsorption of proteins, the Vroman effect, with the most commonly existing blood serum proteins: albumin, immunoglobulin G, and fibrinogen. By presenting the high sensing performance we concluded the L-FBAR as a promising bio-analytical tool.

A C-FBAR was excited in the radial-extensional mode. The shear viscous damping experienced by the C-FBAR was significantly lower than the squeeze damping in L-FBARs, which alleviated the acoustic energy dissipation and increased the $Q$. Removing any complexity of microfluidic channel, $Q$ of up to 189 in water was experimentally demonstrated, which was at least 13-19 times higher than the L-FBAR in liquid with semi-infinite thickness. C-FBAR showed sensitivity to the viscosity of the coupled liquid, and was developed as a viscosity sensor. The C-FBAR was characterized by controlled glycerine solutions to map its resonant frequency changes to viscosity. Experimental results showed very good agreement with the theoretical numbers at low viscosity regime, but found discrepancy when the viscosity exceeded 20 cP, due to the relaxation effect in high viscosity fluid. *In-vitro* coagulation of human whole blood was monitored using the C-FBAR in real-time and as a continuous act, as the viscosity change is an important measure during the clotting process. Coagulation with different initiating conditions was measured as a resonant frequency plot on time scale, in which the points of frequency shift start and saturation indicated the start and end of the fibrin generation; and the overall frequency shift quantitatively revealed the clotting strength.
5.2 Suggestions for future work

Processing of AlN is CMOS compatible which brings out the attempt of replacing ZnO with AlN in a L-FBAR. The major problems of AlN are availability of vendors and the etching difficulties. The latter could be possibly addressed by ion milling. Other advantages of AlN over ZnO include very large electrical resistance, higher intrinsic $Q$, and better resistance to moisture than ZnO. These combined advantages make the migration specially rewarding.

Another room for this work can be to form a sensor array. Combining with pattern recognition techniques, FBAR sensor array can provide fast readout, multi-recognition and thus find systematic level sensing applications.

Device optimization remains for both L-FBARs and C-FBARs. L-FBAR demands a balance between the performance and fabrication complexities. Extremely thin microfluidic channel brings higher $Q$ but also introduces difficulties in a fluid flow (it is easy to be pulled but cannot be pushed) and fabrication challenges. A major challenge for the current C-FBAR is the structural weakness. As the main component of a C-FBAR, the piezoelectric ring is suspended and connected both electrically and mechanically to the substrate via one or a few anchor beams, which are very fragile. Multi-anchor structure addressed this problem but also raised the anchor loss. To reduce the form factor of the C-FBARs seems very promising – it not only increases the device robustness, but also enhances the sensitivities. The only concern is the fabrication difficulty and cost.
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