Micro-electromechanical film bulk acoustic sensor for plasma and whole blood coagulation monitoring

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Abstract

Monitoring blood coagulation is an important issue in the surgeries and the treatment of cardiovascular diseases. In this work, we reported a novel strategy for the blood coagulation monitoring based on a micro-electromechanical film bulk acoustic resonator. The resonator was excited by a lateral electric field and operated under the shear mode with a frequency of 1.9 GHz. According to the apparent step-ladder curves of the frequency response to the change of blood viscoelasticity, the coagulation time (prothrombin time) and the coagulation kinetics were measured with the sample consumption of only 1 μ l. The procoagulant activity of thromboplastin and the anticoagulant effect of heparin on the blood coagulation process were illustrated exemplarily. The measured prothrombin times showed a good linear correlation with $R^2 = 0.99969$ and a consistency with the coefficient of variation less than 5% compared with the commercial coagulometer. The proposed film bulk acoustic sensor, which has the advantages of small size, light weight, low cost, simple operation and little sample consumption, is a promising device for miniaturized, online and automated analytical system for routine diagnostics of hemostatic status and personal health monitoring.

Keywords:

Acoustic resonator; film bulk acoustic resonator; blood coagulation; micro-electromechanical system; viscosity sensor

1. Introduction

Monitoring of blood coagulation is an integral part in the treatment of patients with cardiovascular problems and surgeries when blood gets into contact with artificial surface. For instance, in the surgical procedures employing cardiopulmonary bypass as well as for patients requiring dialysis treatment, extracorporeal circulation of blood is regularly used in conjunction with anticoagulants. These treatments make it necessary to frequently monitor the blood coagulation parameters to keep a sufficient level of anticoagulation in order to decrease the risk of excessive bleeding. In addition, the oral anticoagulant drugs are widely used for the therapy atrial fibrillation, pulmonary embolism, phlebothrombosis and other diseases. For the patients, it is very important to keep the blood coagulation parameters on an appropriate level in daily life to avoid the risk of thrombus or bleeding. In the hospitals, the coagulation parameters in different assays are usually measured by thromboelastography (TEG), paramagnetic particle methods or optical methods (Harris et al. 2013). Although these technologies can offer precise and standardized results, they require complicated instrumentations and professional operators. Moreover, the analytical devices currently on the market necessitate the manual transfer of blood samples from the patient to separate devices and can not permit continuous sampling and real-time measuring. Therefore, there is an urgent need for the miniaturized, online and automated analytical system for the routine diagnostics of hemostatic status and personal health monitoring.

Acoustic biosensors, represented by a quartz crystal microbalance (QCM), have been

used to measure the viscosity change during the coagulation process (Duner et al. 2013; Efremov et al. 2013; Hussain et al. 2015; Jin et al. 2013; Müller et al. 2010). However, the typical QCM device has the size of dozens of square millimeter and the frequency of several megahertz (Beckera and Cooper 2011). Further miniaturization of QCM devices are limited by the capability of traditional mechanical cutting and assembling method. Over the past decade, micro- and nanomechanical sensors, represented by microcantilevers, have been developed for the environment, chemical, and biological detections (Anja et al. 2011; Eom et al. 2011; Waggoner and Craighead 2007). The mass resolutions were achieved to zeptogram and nanogram when the nanomechanical resonators operated in vacuum and fluid environment, respectively (Arlett et al. 2011). Recently, film bulk acoustic resonator (FBAR) has become another promising micro-electromechanical candidate as an alternative to QCM for the gas detection (Chen et al. 2011b; Lu et al. 2015) and bio-sensing applications (Pang et al. 2012; Wang et al. 2014). FBAR features a 1-2 micron-thick piezoelectric thin film, such as AlN (Wingqvist 2010), ZnO (Flewitt et al. 2015), Pb(Zr,Ti)O₃ (Zinck et al. 2005), Ba_xSr_{1-x}TiO₃ (Lee and Mortazawi 2016), and allows a significantly higher frequency and sensitivity than those of QCMs (Voiculescu and Nordin 2012). As an electroacoustic device, the resonant states of FBAR can be affected by the damping of adjacent medium when the device works in the liquid environment, which makes it feasible to monitor the viscoelasticity changes during the biological reaction (Chen et al. 2013; Xu et al. 2012). The size of the FBAR device is within 1 mm², which remarkably reduces the form factor and

manufacturing cost by the batch fabrication based on micro-electromechanical system (MEMS) technology. Furthermore, the miniaturization of the device can handle an extremely small sample volume and perform high throughput tests for the biochemical sensing applications. The disadvantage of the FBAR device is that the device is hardly utilized repeatedly because the material absorbed on the device surface after several times tests is difficult to be removed. As a result, the FBAR devices are seen as disposable sensors for the biochemical applications (Flewitt et al. 2014).

In this study, we present a lateral field excited FBAR for the monitoring of blood coagulation. A portable testing system consisted of oscillators, frequency signal processing and readout circuits was utilized with the availability of direct digital signals. The sequential coagulation stages and the prothrombin times (PT) of plasma and whole-blood samples were measured through the frequency response. The precision and practicability of the proposed FBAR sensor was evaluated by a commercial coagulometer. The procoagulant activity of thromboplastin and the effect of a typical anticoagulant drug (heparin) on the blood coagulation was exemplarily shown.

2. Materials and methods

2.1. Reagents

Blood samples were collected from an apparently healthy donor by the finger-stick method at the clinical laboratory of Huangdao district hospital, Qingdao, China. The whole-blood samples were treated with 3.8% sodium citrate. The plasma samples

were obtained from the whole blood by centrifugation at 3000 rpm for 10 min. The blood samples were stored at 4 °C before use. Rabbit brain thromboplastin (TP) was provided by an instrument vendor of coagulometer, Punlong Medical Equipment Co., LTD (Nanjing, China). The TP concentration used in the experiments was given in arbitrary units per ml (unit/ml); one unit/ml corresponds to a 1000-fold dilution of the pooled TP. CaCl₂ and heparin solution were purchased from Sinopharm Chemical Reagent Co., LTD (Shanghai, China). All reagents were stored refrigerated before use.

2.2 *The configuration of the FBAR sensor*

Fig. 1 shows the lateral field excited FBAR sensor and the testing system for the monitoring of blood coagulation. The sensor was fabricated using a standard MEMS process. As shown in Fig. 1 (a), A 1.5- μ m-thick piezoelectric AlN film was built on an acoustic Bragg reflector consisting of three-period alternating layers of SiO₂ and W with the thickness of one quarter wavelength (0.46 and 0.35 μ m, respectively). The 100-nm-thick Au electrodes deposited on the AlN film were designed to be paralleled with the gap of 5 μ m to generate a lateral electric field. A stable shear mode resonance was excited by the lateral electric field with the frequency of 1.94 GHz and the Q factor of 430 in water (Fig. S1). A testing pool with the dimensions of 6 mm $\times$ 3 mm $\times$ 0.5 mm was fabricated with the sidewall of PDMS to make the resonator surface have a good contact with the liquids. Instead of using SiO₂/AlN as the Bragg reflector in the literature (Chen et al. 2011a), it was used SiO₂/W Bragg reflector due to the smoother surface (decrease of 54% of the roughness, Fig. S2), which reduced the

wave scattering and improved the Q factor of the device (from 313 to 430).

Figure 1

2.3 The portable testing system of the FBAR sensor

In the testing system as shown in Fig 1 (b) and (c), the differential frequency processing was used to minimize the environmental disturbance. For this purpose, two identical FBAR resonators were assembled on the system as the detector and the reference, respectively. The testing liquid was only loaded on the detector device resulting in a frequency difference between the detector and unloaded reference resonator. The details of the circuit design were given in the Fig. S3. In the output spectrum of the FBAR oscillator (Fig. S4), the amplitude of the resonant peak was -3.439 dBm at 1.945 GHz and the average frequency noise produced by the oscillator circuit was about 0.2 dBm. The frequency stability and resolution are related to the noise level. In the FBAR sensor, the noise mostly derived from the thermal noise, 1/f noise due to the quantum fluctuation and phonon scattering by defects, and the wire bonding noise between the device electrodes and the circuit board (Ferre-Pikal et al. 2001).

2.4 The experimental procedures

At first, the detector and the reference FBAR devices worked at same frequency. For the coagulation monitoring, 10 μ l citrated plasma or whole-blood samples, 1 μ l thromboplastin solution and 1 μ l 25 mM CaCl_2 were thoroughly mixed. Then, a droplet of 1 μ l mixture solution was dripped on the surface of detector device,

resulting in a change in the resonant frequency. Then, a droplet of 1 μ l mixture solution was dripped on the surface of detector device, and the reference device with same resonant frequency was kept at an unloaded state. The resonant frequency change of the sample was recorded. The frequency difference of the two devices was used as the response of the testing system. After each coagulation measurement, the device surface was scrubbed using 3% hydrogen peroxide solution and subsequently 10% oxalic acid solution. Then the resonant frequency was measured and compared with its intrinsic value to evaluate the reusability of the device (Fig. S5). After four times of measurements, the changes of the resonant frequency were found to be less than 20 kHz. These deviations can be ignored because that the total frequency downshift due to blood coagulation was more than 8.5 MHz. However, more reused times resulted in an obvious decrease in the resonant frequency due to the unremoved fibrin on the device surface. As a consequence, each FBAR device was used four times in the experiments.

A commercial mechanical coagulometer based on the double magnetic beads method (PUN-2048, Punlong Medical equipment co., LTD, Nanjing, China) was used to evaluate the precision of the proposed sensor. The whole blood, plasma and user's specific samples can be assayed using this coagulometer. The method is immune to the turbidity disturbance and satisfies the requirements of the various test conditions in our experiments. All the experiments were conducted at room temperature (22 °C). The blood samples were restored to room temperature before each measurement to avoid the temperature effect on the viscosity of the blood.

3. Results

3.1 Viscosity characterization of the FBAR sensor

The damping effects on the acoustic resonator depend on viscosity and density of the liquids loaded on the surface of the piezoelectric layer. The glycerol solutions are well suitable to characterize the viscous damping effects on the acoustic devices because its density varies hardly with the concentration (Martin et al. 1993). According to the theory proposed by Keiji Kanazawa *et al.* (Keiji Kanazawa and Gordon Li 1985), the frequency shift of an acoustic resonator is linearly proportional to the value of $\sqrt{\rho_l \eta_l}$ in Newtonian liquids as follows:

$$\Delta f_N = -f_R^{3/2} \sqrt{\frac{\rho_l \eta_l}{\rho_0 \mu_0 \pi}} \quad (1)$$

where Δf_N is the frequency shift in the Newtonian liquid; ρ_l and η_l are the mass density and the viscosity of the loading liquids, respectively; f_R is the resonant frequency in air; ρ_0 and μ_0 denote the density and elastic modulus of the piezoelectric layer, respectively.

The aqueous glycerin solutions with the percent weight of 0-90% were used to characterize the viscosity response of the FBAR sensor as shown in Fig. 2, of which the frequency difference of the reference device and the detector device was used as the response and the error bars are standard deviation (S. D.) of the ten times measurements. In the range of from 1 to 8.64 kg·m⁻²s^{1/2} for $\sqrt{\rho_l \eta_l}$ (glycerin concentration 0-80%), a linear frequency-viscosity dependence with the slope of approximate 337 kHz·m² s^{1/2}/kg was observed, which agrees well with the Newtonian fluid model of the acoustic device. The frequency shift of thickest aqueous glycerin concentration (90%) deviates from the linear relationship, suggesting that the high

viscosity glycerol loaded on the resonator surface can not be considered as a Newtonian liquid and its behavior becomes Maxwellian viscoelastic (Martin et al. 1993). In this situation, the frequency shift is related to the dissipation (D) and can be simplified to the following (Parlak et al. 2013):

$$\Delta f_M = \Delta f_N + \frac{f_R \Delta D}{2} \quad (2)$$

where

$$\Delta D \approx -2 f_R^{1/2} \sqrt{\frac{\rho_l \eta_l}{\rho_0 \mu_0 \pi}} \quad (3)$$

ΔD is the dissipation change due to the strong fluid effect. In this experiment, the value of ΔD can be estimated to be 1.2×10^{-3} for the 90% glycerin solutions.

Figure 2

3.2 The blood coagulation monitoring

The blood samples were activated by homogenously distributed calcium ions and TP. In the extrinsic pathway of coagulation (Efremov et al. 2013), TP (tissue factor) activates factor VII, which in turn leads to an activation of factor X. Together with activated factor V, calcium ions and phospholipids activated factor X forms the so-called prothrombinase complex. Finally, this complex catalyzes the conversion of prothrombin to thrombin. Thrombin as a central protein in the coagulation cascade cleaves fibrinogen to fibrin. During the polymerization and growth of fibrin network, the blood viscoelasticity changes (Müller et al. 2010).

In this work, five plasma samples containing various levels of concentration of TP (0-100 unit/ml) were studied. After the samples were separately dropped onto the sensor, the frequencies decreased about 7.5 MHz immediately due to the sudden loading and viscosity changes (Fig. S6). In order to clarify the details of the frequency behavior during the blood coagulation reaction, the time-dependent frequency profiles

after the plasma and whole-blood samples dropping were shown in Fig. 3. The coagulation responses were similar in the shape to those of QCM (Vikinge et al. 2000b) and contour-mode FBAR (Xu et al. 2012). In the case of no TP, the frequency nearly kept in a constant value over a long time after the sudden frequency falling. In contrast, for both the plasma and whole-blood samples mixed with TP, the frequencies remained almost constant firstly and then gradually dropped to a relatively low value. The initial plateau time and descending time in the frequency responses were extremely dependent on the TP concentration. The more TP contained, the less step-ladder curve resulted owing to the fast coagulation reaction.

Figure 3

The frequency changes revealed the sequential stages of the coagulation reaction. At first, the prothrombin converted into thrombin under the action of thromboplastin. The thrombin as central protein cleaved fibrinogen to fibrin. During this enzyme cascade reaction, the blood viscoelasticity, and thus the resonant frequency, were not changed (Müller et al. 2010). Then, with the polymerization of fibrin, the liquid viscosity increased and more fibrin fibers contacted with the resonator surface, resulting in a continuous decline in the resonant frequency. Finally, a steady state with no further changes reached after the completion of fibrin network.

3.3 The test of PT values

The PT value is an important parameter for the activity of coagulation factors in the extrinsic pathway and the most sensitive indicator for the anticoagulant therapy. After the activation of thromboplastin and calcium ions, the PT values of the blood samples can be determined as the end of fibrin polymerization. As a novel method for coagulation analysis, it is essential to choose a characteristic point in the frequency

response to determine the PT value. For this purpose, as shown in Fig. 3, the first stable frequency after sample dropping (frequency difference ~ 7.5 MHz) was defined as the base line to evaluate the decrease of frequency for each TP concentration. The times when the frequency decreased to three typical percentages (90%, 95%, 100%) of the maximum downshift of frequency (Δf) were compared with the results acquired by the commercial coagulometer (Tab. S1). The results showed that the times when the frequency decreased to 95% were nearest to the PT values measured by the coagulometer. Consequently, these characteristic points were determined as the PT values of the blood samples as summarized in Tab. 1. As expected, more TP in the sample caused shorter coagulation time, which were in the range of 130~700 s. The coefficient of variation (C. V.) between the two methods was less than 5%. In addition, the frequency responses to the same volume of TP solutions without blood were also measured (Fig. S7). It was found that the base frequency line was not affected by the TP concentration.

Table 1

3.4 A Comparison with commercial coagulometer

In order to evaluate the practicability of the proposed biosensor, a commercial coagulometer was used to measure the coagulation time and compare with the FBAR sensor. The correlation and consistency of the two methods were shown in Fig. 4. As shown in Fig. 4 (a), a linear relationship was found between the two methods with the regression equation of $y=0.994x+3.701$ ($R^2=0.99969$). In both science and clinical research, a well-established procedure for the comparison of two different

measurement techniques is Bland-Altman plot. This type of diagram was used to compare our proposed sensor with the commercial coagulometer as shown in Fig. 4 (b). The value differences of the two methods were plotted against the mean of the corresponding values. One can see that all data points lied in the given range of mean $\pm 2S.D.$ with a symmetrical distribution, which declare that about 95% of the values were within two standard deviations. The data in the Bland-Altman plot showed that none of the methods delivered higher or lower values. The measurement consistency of the FBAR sensor with the well-approved method illustrated that the proposed sensor can be used as a feasible tool to analyze blood coagulation.

Figure 4

3.5 Heparin sensitivity test

In both laboratorial blood testing and clinical therapy, heparin is a widely used anticoagulant drug (Freedman 1992). Heparin binding to thrombin alters the affinity towards fibrinogens, which weakens the fibrin generation and therefore slows down the coagulation process (Kaatze 2010). Here we used the FBAR sensor to demonstrate the anticoagulant effect of the heparin by adding 5 μ l heparin solution to the plasma samples (activated by 50 unit/ml TP). Fig. 5 shows the time-dependent resonant frequency profiles at the heparin concentrations of 0.2-1 IU/ml. Obviously, the addition of heparin resulted in an increase of coagulation time, which showed very good accordance with the FBAR sensor and the commercial coagulometer (Fig. S8). In addition, when excess quantity of heparin was added in the blood sample, the both methods showed the absence of coagulation process during the test time. These results clearly indicated the anticoagulant effect of heparin on the blood coagulation.

Figure 5

3.6 The kinetics of blood coagulation

In order to reveal the coagulation kinetics, the time-dependent frequency profile of the FBAR sensor was fitted using a sigmoid curve (Evans et al. 2008; Jain et al. 2016; Vikinge et al. 2000a) as follows (Fig. S9):

$$f(t) = \frac{\Delta f}{1 + e^{-2k(t-t_{tag})}} \quad (4)$$

where $f(t)$ is the frequency response at time t , Δf is the maximum frequency downshift, k is the reaction rate constant, and t_{tag} is the time at which the frequency equals the half value of Δf .

The extracted kinetics parameters were listed in Tab. S2 and Tab. S3. As expected, the blood sample activated by higher concentration TP had a higher reaction rate constant k (Fig. S10 a). According to the gel architecture proposed by Blombäck *et al.* (Blombäck et al. 1994), the fibrin network structure is determined by the initial coagulation rate, which is related to the concentration of activator and fibrinogen. A higher activator concentration gives a greater number of nucleation sites from which the fibrinogen monomers can start the polymerization, thus giving a higher polymerization rate than that at a lower activator concentration. In addition, the presence of heparin reduced the coagulation time and resulted in a low reaction rate constant (Fig. S10 b).

The blood viscoelastic properties and coagulation kinetics are influenced by the hematocrit, morphology of the red cell and the concentration levels of cholesterol,

triglyceride and fibrinogen. The diet, habit, health status and usage of medicine have a significant impact on the human blood viscoelasticity and coagulation kinetics. The frequency responses of the FBAR sensor to the blood coagulation are also influenced by these factors. In the further studies, a large sample database need to be established for the new technology.

4. Discussion

The uncertainty of the system is closely related to the frequency resolution and the noise of the FBAR sensor. The frequency resolution R was estimated to be about 30 Hz from the frequency fluctuation at the based resonant frequency f_0 as follows (Rodriguez-Pardo et al. 2004)

$$R = \sigma(\tau) \cdot f_0$$

where $\sigma(\tau)$ is the Allan variance based upon the resonant frequency measurements over time (Fig. S11).

In the realized testing system, two identical FBAR devices were used. One measured the change of blood viscoelasticity, while the other one detected the background resonance that were subtracted to provide a better signal. In consideration of the requirement of linear output, the minimum detectable frequency of the Barron transformer was designed to 20 kHz, which is enough to distinguish the frequency changes due to the blood coagulation. As shown in the time-dependent frequency profiles (Fig. 3), the average noise level and the signal-to-noise ratio were estimated to be 36 kHz and 208, respectively.

According to theoretical model of acoustic resonator, the shear wave penetration

depth is 200-300 nm in the blood (Keiji Kanazawa and Gordon Li 1985). That is to say that the sample volume suffered the shear perturbations is very small; much smaller than, for example, the typical size of red cell. The oscillations occurring close to the surface cannot actually perturb blood cells until they are attached (Efremov et al. 2013). Furthermore, the blood proteins, especially fibrinogen and fibrin fibers, behave not viscously but rigidly after their attachment for a wide range of coating materials (Andersson et al. 2005). When the blood start to clot, not only the change in viscosity but also the change in elasticity and the fibrin adhesion may occur simultaneously as the result of the formation of fibrin fibers (Lakshmanan et al. 2014). In addition, other factors, such as the nonhomogeneous shear vibration, boundary effect and micro-form standing wave for the ultra-small sample volume, may influence the resonant state of the FBAR sensors. Actually, the frequency shifts of the FBAR sensor as shown in Fig. 3 were determined by the comprehensive viscoelasticity changes during the blood coagulation instead of only the absolute viscosity, which limits the application of the FBAR sensors in the measurement of blood viscosity. A detail theoretical model to predict the effects of viscosity and other factors on the FBAR device will be studied in our further work.

As shown in Fig. 3 (a), the maximum frequency downshift Δf was observed decreased with the increase of TP concentration. The dependence of the maximum response on the activator concentration was in agreement with the results obtained from QCMs (Chang et al. 2000; Cheng et al. 1998; Vikinge et al. 2000b), SPR (Vikinge et al. 2000a) and the shear gradient-activated microfluidic device (Jain et al.

2016). This behavior is in accordance with the fact that the fibrin fibers grow thicker and longer as the increase of coagulation time (Efremov et al. 2014). At a constant fibrinogen concentration, the activator concentration determines the number of nucleation sites. The fiber formation from fewer nucleation sites leads to fewer, but thicker, rigid and longer fibers at the low activator concentration (Blombäck et al. 1994). In contrast, the rapid coagulation process, at high activator concentration, leads to many thin and small fibrin fibers that expose a relative larger area to the surrounding liquid and thus are considered more flexible (Efremov et al. 2014). As a consequence, the low-density network results in a less frequency shift at the high TP concentration as shown in Fig. 3 (a), which is also in agreement with the microstructure of the fibrin network observed by Viking *et al.* (Viking et al. 2000a) and Lakshmanan *et al.* (Lakshmanan et al. 2016). Their results showed that the fibers were shorter, wider and fewer in number as a result of slow formation rate in the presence of low TF concentration while the fibers became longer, thinner and less numerous due to the more rapid onset of clot formation at higher TF concentrations. Moreover, for the whole-blood samples, the frequency behaviors showed no obvious dependence on the TP concentration, although the coagulation times did. The main difference between the whole blood and plasma is the presence of blood platelets. As an important modulators of blood coagulation in vivo, platelets provide activation and nucleation sites for the fibrin polymerization (Cao et al. 2007). Compared to the plasma, the effect of TP on the buildup of fibrin network may be modified due to the incorporation of platelets and other blood cells on the fibrin fibers

(Wu et al. 2016).

The blood coagulation monitoring device described here provides several potential advantages over other analytical devices. First, the FBAR device can be mass-manufactured on wafer level at low cost utilizing the existing silicon micro-electronics process, which is an essential requirement for single-use disposability. As a comparison, the market price of the FBAR filter chip which Avago shipped in 2006 has been reduced to less than 2 dollar in the recent years (Ruby et al. 2012). Another promising advantage is the feasibility of the integration of the FBAR sensor array with the CMOS electronics in a single-chip configuration. For example, Siemens has demonstrated an on-chip array composed of 64 pixel CMOS-integrated FBAR devices with the area less than 1 mm×1 mm (Nirschl et al. 2010). The miniaturization of the full system also facilitates to reduce the specimen consumption. This means that each blood test requires a smaller pinprick and hurts less. In this work, the FBAR sensor has the area of only 500 $\mu\text{m} \times 500 \mu\text{m}$ (not including the liquid pool) and the sample consumption of 1 μl , which is much less than that of the existing coagulometer, such as TEG, QCM, turbidimetric and mechanical meter (Harris et al. 2013). In the further work, the existing miniaturized and automatic blood reprocessing module and electronic components can be integrated in the FBAR based system. The cost per test of the FBAR sensor is estimated to be less than 3 dollars considering the equipment depreciation, the fabrication cost of the disposable FBAR devices and the low consumption of the test reagents. This test cost is equivalent to the widely used paramagnetic particle methods and optical methods in laboratories. However, the

FBAR based system is more suitable for the personal health monitoring thanks to its compactness, portability, quick readout and simple operation.

5. Conclusions

We have demonstrated that the MEMS based lateral field excited FBAR is a feasible tool for studying the rheology of blood. The FBAR working under shear mode was characterized by controlled glycerin solutions to map the frequency-viscosity relation and then used to monitor the coagulation process of the plasma and whole-blood samples. The frequency response had an apparent step-ladder curve that was distinctly suitable for determining the coagulation time. The procoagulant activity of thromboplastin on the coagulation process were obvious. The testing results showed a good consistency between the FBAR sensor and a commercial coagulometer. Significantly, we were able to employ a small size, light weight, low cost and simple operation to realize the blood coagulation. Only one microliter of blood sample was required for the proposed sensor. The statistical data has proven the precision of the sensor for clinical application. Furthermore, with the integration of MEMS manufacturing techniques, the FBAR sensor offers a high potential for developing miniaturized, completely online and automated analytical system for routine diagnostics of hemostatic status in surgical operations and personal health monitoring. In our further study, we will conduct measurement series toward using pathological and clinically relevant blood samples.

Acknowledgements

This work was supported by National Science Foundations of China (No. 61574086, No. 11504207), National Science Foundation of Shandong Province No. ZR2014AQ006, Qingdao science and technology program of basic research projects [15-9-1-78-jch], Project of Shandong Province Higher Educational Science and Technology Program [J15LJ0]. The authors acknowledge the clinical laboratory of Huangdao district hospital, Qingdao, China for the blood sample collecting.

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List of figure captions

Fig. 1 The basic device configuration and the testing system of the FBAR sensor for blood coagulation monitoring. (a) The microphotograph and the cross-section view of the FBAR device; (b) the system diagram; (c) the packaged PCB of the testing system; (d) the photograph of overall system

Fig. 2 The resonant frequency of the lateral field excited FBAR device as the function of the value of $\sqrt{\rho_l \eta_l}$ following in aqueous glycerin solutions. The error bars are standard deviation of the ten times measurements. The frequency difference of the reference device and the detector device is used as the response.

Fig. 3 The time-dependent resonant frequency profiles in the absence and presence of increasing concentration of TP in the blood samples. (a) The plasma samples; (b) the whole-blood samples.

Fig. 4 (a) The correlation of PT values determined by the commercial coagulometry and FBAR sensor. (b) The Bland-Altman plot of the FBAR sensor and the commercial coagulometer. The error bar of the coagulation time is the standard deviation of ten times measurements for each blood sample.

Fig. 5 The time-dependent resonant frequency profiles of the plasma samples mixed with different concentrations of heparin. (a) no heparin; (b) 0.2 IU/ml; (c) 0.4 IU/ml; (d) 1 IU/ml. The plasma samples were activated by 50 unit/ml TP solution.

Tables

Table 1 The measured frequency shift and the PT values of the FBAR sensor for different plasma and whole-blood samples.

		Plasma samples			Whole-blood samples		
Experiment	TP concentration (unit/ml)	Frequency decrease* (MHz)	PT (Sec)	C.V**	Frequency decrease* (MHz)	PT (Sec)	C.V**
1	0	--	No coagulation	--	--	No coagulation	--
2	1	-8.971±0.13	682±16	3.6%	8.714±0.14	722±24	2.8%
3	10	-8.951±0.10	548±15	2.4%	8.821±0.13	565±17	2.6%
4	50	-8.386±0.15	232±6	2.5%	8.759±0.16	262±8	3.2%
5	100	-8.177±0.16	130±4	3.0%	8.715±0.12	168±5	2.3%

* 95% of the maximum downshift of frequency (Δf)

** The coefficient of variation between the results of FBAR sensor and the commercial coagulometer.

Figure 1

(b)

The FBAR sensor

The cross-section view

2 mm

(a)

(c)

(d)

Figure 2

Figure 3

Figure 4

Figure 5