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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 μl. 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.

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 micro-cantilevers, have been developed for the environment, chemical, and

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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 biosensing applications (Pang et al., 2012; Wang et al., 2014). FBAR features a 1–2 µm-thick piezoelectric thin film, such as AlN (Wingqvist, 2010), ZnO (Flewitt et al., 2015), Pb(Zr,Ti)O_3 (Zinck et al., 2005), $Ba_xSr_{1-x}TiO_3$ (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 mm^2, 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 3,000 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. $CaCl_2$ 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-µm-thick piezoelectric AlN film was built on an acoustic Bragg reflector consisting of three-period alternating layers of SiO_2 and W with the thickness of one quarter wavelength (0.46 and 0.35 µm, respectively). The 100-nm-thick Au electrodes deposited on the AlN film were designed to be paralleled with the gap of 5 µm 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×3 mm×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 SiO_2/AlN as the Bragg reflector in the literature (Chen et al., 2011a), it was used SiO_2/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).

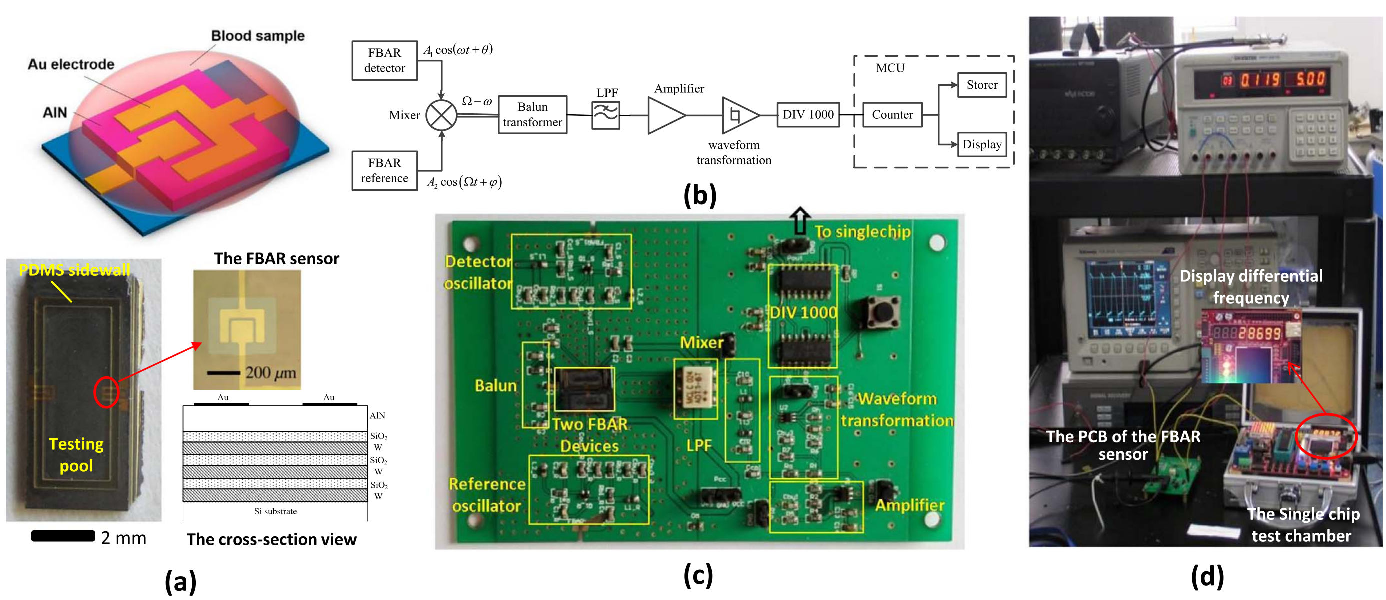

Fig. 1. The basic device configuration and the testing system of the FBAR sensor for blood coagulation monitoring. (a) The microphotograph and the cross-section view of the FBAR device; (b) the system diagram; (c) the packaged PCB of the testing system; (d) the photograph of overall system.