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Label-free detection of pepsinogen 1 and 2 by polyethylene coating Lamb microfluidic device

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ABSTRACT

Early screening of gastric cancer is a critical importance for the improvement of patients' survival rate. Here, a polyethylene coating Lamb (PE-Lamb) microfluidic device with immune layer for gastric cancer label-free detection was constructed. Two serum pepsinogen 1 (PG1) and pepsinogen 2 (PG2) biomarkers were applied to screen and predict the appearance of gastric cancer. Compared with enzyme-linked immunosorbent assay (ELISA), this method achieved a higher sensitivity and less time (40 min vs 120 min). The limit of detections (LOD) were reached 60 pg/mL for PG1 and 30 pg/mL for PG2, which have two orders of magnitude lower than traditional ELISA. The linearity coefficient indexes (R^2) for PG1 and PG2 were 0.992 and 0.953 respectively, which is similar to that of ELISA. In addition, PG1 and PG2 mixed antigens sample with human serum was detected by PE-Lamb approach, and the frequency response showed high reproducibility and specificity. The results indicate that PE-lamb diagnostic technique is a novel and promising method for high-throughput screening and early diagnosis of gastric cancer.

1. Introduction

Serum pepsinogens (PGs) are serum markers of atrophic gastritis, which are regarded as a precancerous change in the stomach (Dinis-Ribeiro et al., 2004; Miki, 2002). Thus, for the detection of gastric cancer, patients at high risk of gastric cancer have been screened clinically by the PGs detection (Abnet et al., 2011; Dinis-Ribeiro et al., 2004; Juan et al., 2017; Miki, 2011; Oishi et al., 2006; Samloff et al., 1982). PGs consist of two biochemically and immunologically distinct types, pepsinogen1 (PG1) and pepsinogen2 (PG2) (Miki et al., 2003). Until now several detection methods and assays have been used to detect PGs, such as radioimmunoassay (RIA) (Ichinose et al., 1982), fluorescent microassay method (Miki et al., 1982), enzyme immunoassays (EIAs) (Yasukawa et al., 2007), chemiluminescence enzyme immunoassay (CLEIA) (Nakagawa et al., 2013), and enzyme-linked immunosorbent assays (ELISA) (Abnet et al., 2011; Huang et al., 1987; Jikihara et al., 1992; Juan et al., 2017). Especially, ELISA is widely used for clinical gastric cancer diagnosis due to its extremely high reliability, specificity, and accuracy, as well as feasibility for large number of samples. However, limitations still remain in these current methods, such as time-consuming and color background interference. In addition to major equipment requiring, luminescent label, vast samples, and experienced personnel. As early screening of gastric cancer is supposed to be accurate, sensitive, rapid, easily-operated and low-cost, it is imperative to develop a novel diagnostic technique.

The label-free device with a high sensitivity and no color background influence for mass detection was obtained by amazing acoustic micro sensor. Different types of acoustic micro sensor, such as Quartz crystal microbalance (QCM) (Pirich et al., 2017), Sezawa-mode surface acoustic wave (SAW), flexural plate-wave (FPW) (Huang et al., 2012) and Lamb have been reported to detect various biomarkers by immunoassay and promising results were obtained. The acoustic micro sensor are reliable, simple equipment needed (Huang et al., 2012; March et al., 2009), especially, they are label-free, no color background influenced and highly sensitive (Chen et al., 2017; Larsen and Hvas, 2017; Nam et al., 2011). However, except for Lamb, most of the

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acoustic micro sensors usually require complicated operation, long testing time and with disadvantages of high noise and low mass sensitivity. In addition, most immunoassays are carried out in a liquid environment and the electrodes is easily short circuited, so that most of the acoustic micro sensors do not suitable to use. Lamb wave sensor's input/ output inter-digital electrodes (IDTs) and AlN piezoelectric layer on different side with the detection cavity, which enabled maintaining the performance of the sensor since the IDTs and AlN layer would neither be contaminated by the sample and reagents, nor short-circuit by the working liquid, so it could be used for immunoassay in biosensing (Huang et al., 2012; Schmitt et al., 2013; Wang et al., 2012). IDTs and AlN piezoelectric layer of the Lamb wave sensor's determine the quality and stability, IDTs and AlN layer can contact with some reagents or sample in some process of the immune layer build and antigens test, which will lead to a change in the electrical properties of the gold IDTs and the corrosion of AlN piezoelectric layers, and the stability and sensitivity of the Lamb wave sensor will be affected.

In order to use the Lamb wave sensor to achieve a high sensitivity, low noise and rapid PGs immunoassay method, the upper surface of IDTs side can be covered to protect IDTs and aluminum nitride (AlN) layer from being contaminated and corroded to improve Lamb wave sensor's stability with a waterproof, insulating, thermoplastic, and transparent polymer coating (Hoffman and Miller, 1997) layer, which is an effective way to avoid contact between reagents and IDTs or AlN layer.

In this research, polyethylene (PE) coating was adopted to maintain the Lamb sensor in good stability during the functionalization and test steps, which a significant increase in the quality factor of Lamb wave sensor in PGs immunoassay. In our design, polyethylene covered Lamb (hereafter named as PE-Lamb) microfluidic device was put forward with a microfluidic chip and PE coating Lamb wave sensor, which sensor has PE coating layer to protect the IDTs and an immune layer. The immune layer was immobilized with antibodies on gold surfaces of detection cavities by self-assembling monolayer (SAM). Anti-PG1 and anti-PG2 double antibodies functionalized PE-Lamb array biosensor was constructed to achieve the label-free detection for early screening of gastric cancer. Compared with traditional ELISA, PE-Lamb used less time for PG1 and PG2 test. The limit of detection (LOD) was two orders of magnitude lower than that of traditional ELISA, and linearity was equivalent to that obtained by traditional ELISA.

2. Materials and methods

2.1. Reagents and solutions

Mouse monoclonal anti-PG1 (1 mg/mL), mouse monoclonal anti-PG2 (1 mg/mL) were purchased from Abcam (UK). Human PG1 ELISA Kit and Human PG2 ELISA Kit were purchased from BIOHIT OYJ (Finland), and antigens PG1 and PG2 were from the ELISA Kits. 11-mercaptoundecanoic acid (MUA), 11-Mercapto-1-undecanol (MUD), N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), N-Hydroxysuccinimide (NHS), polyethylene, ethyl alcohol, acetone, vitriol, hydrogen peroxide, hydrochloric acid, toluene, 1-Methylimidazole, Tween20, methylbenzene, Bovine serum albumin (BSA), glycine were purchased from Sigma-Aldrich. The BSA was used to passivate unspecific sites (Hao et al., 2009). 0.01 M Phosphate buffer saline (PBS, pH 7.2) was purchased from GE Healthcare Life Sciences (America) was used to dilute antibodies and antigens. And the PBS solution containing 0.05% (v/v) Tween 20 (PBST) was used for rinse off non-specific bindings and obtain a stable baseline (March et al., 2009). 0.1 M 1-Methylimidazole buffer was prepared and the pH was adjusted to 5.0 by hydrochloric acid and 0.01 M pH 2.6 glycine-HCl buffer was formulated too. All the chemicals were analytical grad. All aqueous solutions were prepared using high-purity distilled, deionized water and were filtered through a 0.22 μm membrane filter immediately prior to use. The glycine-HCl buffer, PBS and PBST solution were handled in −80 KPa for 12 h to eliminate bubble.

2.2. Equipment

Network Analyzer E5061B (Agilent, America) was used for PE-Lamb tests signal detection and acquisition, all the data were collected under S_{21} mode. The setting central frequency and span were ~8.4 MHz and 2 kHz respectively. DT-230A thermostat (Dongkang, China) was used for incubate in the immune response. SynergyHT microplate reader (BioTek, America) was used for comparing results of PE- Lamb tests. Milli-Q filtration system (Nihon Millipore Ltd., America) was used to product the deionized water.

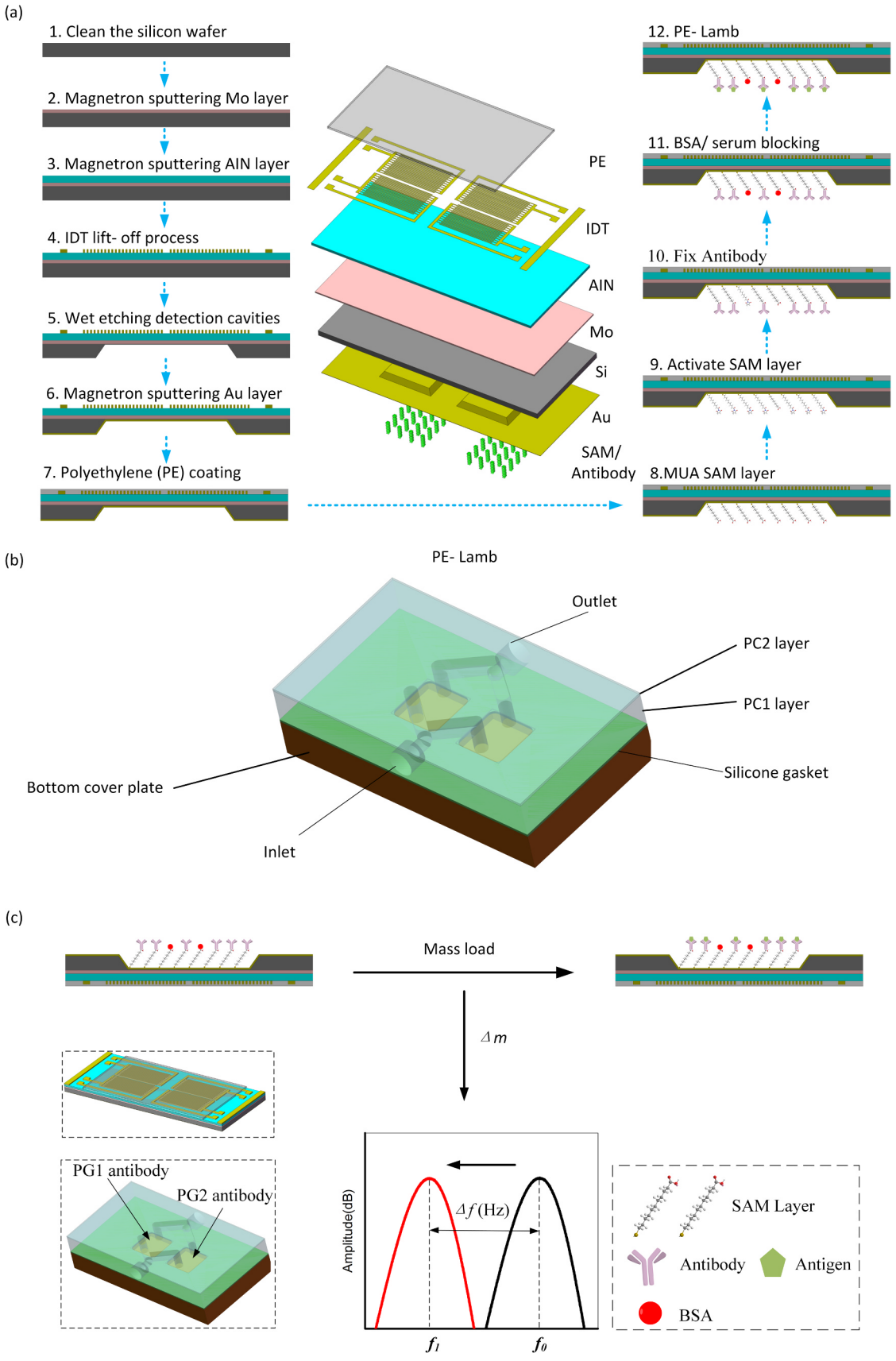
2.3. Fabrication of PE-Lamb

Prior to PE-Lamb sensor formation, 400 μm silicon wafer was cleaned in acetone and ethyl alcohol for 10 min with ultrasonic cleaning, and then rinsed with deionized water, dried in nitrogen. 200 nm Mo layer and 2 μm aluminum nitride (AlN) layer were deposited on one side of the silicon wafer by magnetron sputtering subsequently. Two 12 pairs of 120 nm gold inter-digital electrodes (IDTs) were made, the width and gap of the fingers were 50 μm was deposited by lift-off process. Two 4 mm × 4 mm cavity arrays in the other side of the sensor were corroded by KOH solution, which at the corresponding position of the two IDTs, then washed again in acetone and ethyl alcohol, rinsed with deionized water and dried in nitrogen. The 124 nm gold layer was deposited on the side which were corroded cavity arrays by magnetron sputtering subsequently to build the self-assembled monolayer (SAM) and fix antibodies on the surface of the detection cavities. PE was dissolves in toluene and coated on the surface of the side of IDTs by means of spin coating to protect IDTs and AlN layer from contamination and corrosion in sensor functionalization and PGs test steps. The major physical design specification of the PE-Lamb sensor were listed in Table 1. Then the gold layer cleaned by fresh piranha solution (H_2SO_4: 30% H_2O_2 (v/v) was 3:1) for 5 min. Rinsed with deionized water for 5 min and ethyl alcohol for 3 min, dried using nitrogen.

After cleaned up, the sensor was immersed into 80 mM MUA and 60 mM MUD in ethyl alcohol incubated in room temperature for 24 h to form a mixed SAM on the gold surface (Arvand et al., 2018; Frederix et al., 2003; He et al., 2015; Ko et al., 2007; March et al., 2009; Yang et al., 2017). Due to the IDTs has been protected by PE layer, the SAM just formed on cavities' gold surface, which is the main point to realize a PGs immunoassay with Lamb wave sensor. The sensor was washed by 10 mL of ethyl alcohol and deionized water respectively. Subsequently, each of the detection cavity was transferred into 20 μL 200 mM EDC and 25 mM NHS in mixed 0.1 M 1-Methylimidazole-HCl buffer (pH 5.0) and soaked for 15 min to activate the carboxyl of the SAM, followed by washed with 5 mL PBS buffer and dried in nitrogen. Once the carboxyl was activated, 20 μL anti-PG1 and 20 μL anti- PG2 at a concentration of 100 μg/mL were dropped into each detection cavities and incubated for 24 h at 4 °C to let the amino-group of the antibodies react with the carboxyl of the SAM on the detection cavity arrays surface. The weakly

Table 1
Major physical design specification of the Im-Lamb sensor.

Item	Parameter
IDTs finger width/ gap	50 μm/50 μm
Wavelength of IDTs	200 μm
Number of IDTs finger pairs	12 × 2
Detection chamber silicon layer thickness	15 μm
Central frequency	~8.4 MHz
Detecting cavity size	4 mm (L) × 4 mm (W) × 0.394 mm (H)
Number of cavities	2
PE-Lamb sensor size	17 mm (L) × 7 mm (w)



Scheme 1. (a) Schematic diagram of PE- Lamb preparation process; (b) The schematics of the PE- Lamb microfluidic device; (c) Schematic illustration of the principle of PE-Lamb mass detection.

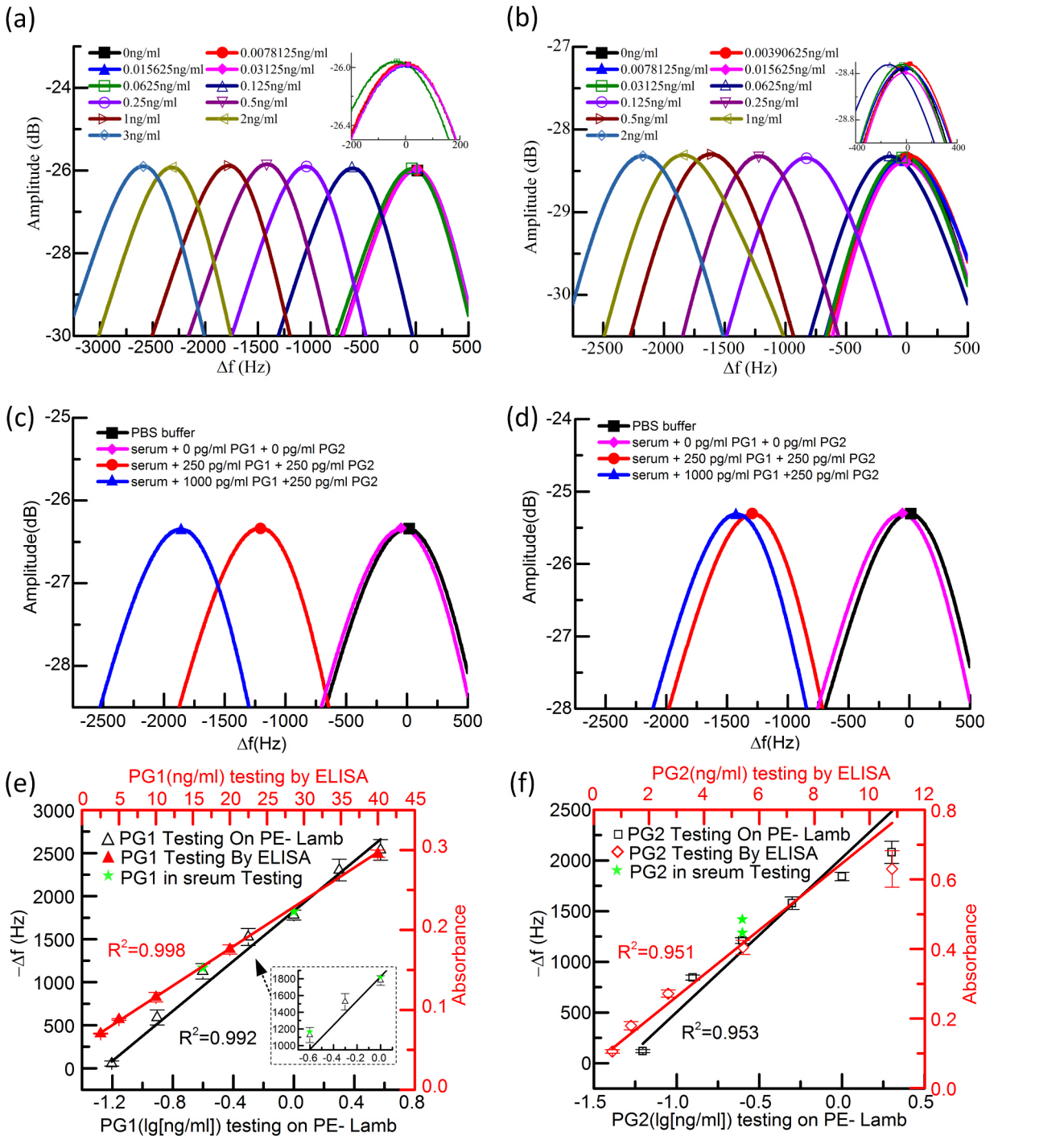


Fig. 1. PG1 and PG2 test on PE-Lamb. (a) The central frequency response with different concentrations PG1 antigen; (b) The central frequency response with different concentrations PG2 antigen; (c) The central frequency response with different concentrations PG1 and PG2 antigen in serum on PG1 antibody fixed cavity; (d) The central frequency response with different concentrations PG1 and the same concentration PG2 antigen in serum on PG2 antibody fixed cavity; (e) The linearity results of different concentrations of PG1 antigens tested by PE-Lamb and ELISA; (f) The linearity results of different concentrations of PG2 antigens tested by PE-Lamb and ELISA.

absorbed or nonspecifically antibody was rinsed up and removed out by 5 mL PBST. 10 mg/mL 20 μL of BSA was subsequently added into the detection cavity arrays to passivate non-specific adsorption sites for 4 h at 4 °C (Blocked by healthy human serum, when PE-Lamb was used to test the serum sample). Then 5 mL PBST was added to remove any weakly adsorbed BSA or serum components. PE-Lamb was prepared, as shown in Scheme 1(a).

The PE-Lamb sensor was embedded into a microfluidic chip, as shown in Scheme 1(b), with five layers. The top cover consists of two Polycarbonate (PC) layers, and the first PC (PC1) layer has inlet and outlet, the second PC (PC2) layer was used for sealing. The bottom cover plate of the microfluidic chip was fabricated with polymethyl methacrylate (PMMA), and 10 shrinkable probes have been built to conduct the signal and connected to a Printed Circuit Board (PCB) which was attached to the bottom of cover plate. The PE- Lamb was sandwiched between top cover plate and bottom cover plate with a silicone gasket in the detection cavities side to ensure only the side of corroded cavity arrays would touch the solution and each of the cavity

volume is about 10 μL, also made sure the PE-Lamb connected with the probes stably.

The developed PE-Lamb sensor is extremely sensitive to the changes of cavities surface mass by monitoring the frequency changes from a Network Analyzer linked to the circuit (Liu et al., 2017). The changes in the mass loading of the surface are transduced into changes of the resonant frequency. The measuring signal is the resulting frequency shift due to the mass deposition Δm of an analyte on the surface as shown in Scheme 1(c). The mass deposition depending on concentration of analyte.

2.4. Standard antigens detection on PE-Lamb

Before antigens test, a stable central frequency was acquired at 37 °C without loading any solution. The central frequency fluctuation in air needs to be limited within less than ± 1 Hz in 60 s. Meanwhile, different concentrations of standard PG1 and PG2 antigens mixed solutions, PBST and Glycine-HCl solution (pH 2.6) were incubated at 37 °C in water bath. Then 30 μL pre-incubated different concentrations of standard PG1 and PG2 antigens mixed solutions were pumped into the PE-Lamb microfluidic device and each concentrations of the antigens solutions were incubated with the PE-Lamb for 40 min at 37 °C. Subsequently, the 180 μL pre-incubated PBST was then pumped again, to avoid non-specific binding of antigens, the central frequency fluctuation was also required less than ± 1 Hz in 60 s, and this step was also repeated three times and the central frequency was recorded. The PE-Lamb sensor was taken out and dried in nitrogen, and was put into the microfluidic device again and detected after the PE-Lamb reached 37 °C when the central frequency fluctuation less than ± 1 Hz in 60 s. In order to regenerate PE-Lamb, before next concentration was pumped, 50 μL pre-incubated Glycine-HCl solution was pumped into the solution to release antigen-antibody complexes from the SAM surface (Drake and Klakamp, 2011; Tang et al., 2006). Then 180 μL pre-incubated PBST was pumped into the solution to eliminate Glycine-HCl. Repeat to pump Glycine-HCl solution and PBST alternately for 3 times to regenerate PE-Lamb. Other concentrations of standard PG1 and PG2 antigens mixed solutions detection same as the above method. Each of the optional, the flow rate of the pump is 60 μL/min, and the central frequency response were recorded with the network analyzer with dry PE-Lamb sensor.

2.5. Serum dilution antigen detection on PE-Lamb

The healthy human serum after 300 times diluent was used as dilution to formulate different concentrations of PG1 and PG2 antigens mixed solutions, and the detection same as the above method.

2.6. Antigens detection by ELISA

ELISA was considered as one of the commonly used methods of clinical testing, and is widely used in detection of specific antibodies and antigens (Xie et al., 2015; Yasukawa et al., 2007). The different concentrations of standard PG1 and PG2 antigens mixed solutions were tested by ELISA to compare with PE-Lamb.

2.7. Control experiments on PE-Lamb

PE-Lamb sensors without the antibodies at the surface of detection cavities, which were fabricated as the processes were shown in Fig. 1(a) without the step 10. Standard antigens were tested on the PE-Lamb without antibodies and the activated SAM layer has been blocked by 10 mg/mL 20 μL of BSA. The detection same as the above method in 2.4.

2.8. Specificity and interference experiments on PE-Lamb

Different concentrations of single PG1 or PG2 standard antigens in PBS buffer were detected to explain the specificity and anti-interference of PE-Lamb. PE-Lamb were fabricated with anti-PG1 and anti-PG2 on the surface of the detection cavities and the PG1 or PG2 antigens were tested with the method, which was the same as 2.4.

3. Results

3.1. Evaluation of PE-Lamb performance

Quality (Q) factor value was one of essential parameters contributes to PE-Lamb (Lin et al., 2011; Lu et al., 2007), and the high Q factor is one key factor contribute for the measurements precision, indicated effective signal quality. There are several peaks in PE-Lamb, which is the effect of reflections of Lamb waves that produce several resonances, and the largest Q factor peak was chosen in this study, and the Q factor value was about 4676. The effect of multiple reflections is main reason for high Q factor. Q factor is decided by the length of Lamb wave membrane, the numbers of IDTs, the reflection coefficient of at the edge of membrane (Lefevre et al., 2009; Zhou et al., 2009). It is convincing to compare the Q factor of PE-Lamb with traditional Lamb wave sensor. Q factor was increased by 5.19% ± 0.62% in 5 times in sample test. The results implied that the increase of Q factor value might be caused by PE coating layer which protected IDTs and AlN layer from being contaminated or corroded by reagents or samples to improving the stability. In addition, PE coating layer made the upper surface of PE-Lamb smoother than traditional Lamb, which reduced the energy loss in IDT fingers (Zhou et al., 2009). The largest Q factor peaks were fund and used in this study, which lead to high sensitivity and resolution. Therefore, PE-Lamb was available for repeatable PGs measurements for early screening of gastric cancer.

3.2. PGs testing on PE-Lamb

Different concentrations of standard PG1 and PG2 were detected according to Table 2. The PE-Lamb responses to each concentration of PG1 and PG2 and the central frequency changes were recorded after the PE-Lamb was dried by nitrogen. The central frequency response with standard PG1 and PG2 antigens mixed sample on anti-PG1 modified cavity is shown in Fig. 1(a), and response on anti-PG1 modified cavity is shown in Fig. 1(b). The PE-Lamb's LOD of standard PG1 antigen in PBS buffer test was 0.06 ng/mL, and standard PG2 antigen in PBS buffer test was 0.03 ng/mL, respectively. EIAs was reported in the LOD for both PG1 and PG2 were 1.6 ng/mL (Yasukawa et al., 2007). Electrochemical

Table 2
The different concentrations of standard antigens in PBS.

Antigens	Concentrations gradient (pg/mL)										
	PE-Lamb										
PG1	0	7.81	15.62	31.25	62.50	125	250	500	1000	2000	3000
PG2	0	3.90	7.81	15.62	31.25	62.50	125	250	500	1000	2000
	ELISA										
PG1	0	650	1250	2500	5000	10,000	20,000	40,000	–	–	–
PG2	0	170	340	680	1360	2720	5440	10,880	–	–	–

Table 3
Comparison of the proposed PE-Lamb with recently reported methods.

Techniques	Detection range (ng/mL)		Detection limit (ng/mL)		Ref
	PG1	PG2	PG1	PG2	
Electrochemistry	37.5–600	2.5–80	37.5	2.5	(Xie et al., 2015)
EIAs	1.6–60.3	1.6–60.3	1.6	1.6	(Yasukawa et al., 2007)
CLEIA	3.65–192.73	0.56–53.01	0.412	0.497	(Cho et al., 2016)
TRFIA	0.33–300	0.38–40	0.33	0.38	(Fan et al., 2017)
Immunoassay strip	0.5–500	0.5–500	0.5	0.5	(Wu et al., 2017)
PE-Lamb	0.06–3	0.03–2	0.06	0.03	This work

microfluidic chip was used to have the linear ranges of detection were 37.5–600 ng/mL for PG1 and 2.5–80 ng/mL for PG2 (Xie et al., 2015).

The linearity analysis results of 11 concentrations of each PG1 and PG2 antigens tested by PE-Lamb are shown in Fig. 1(e) and Fig. 1(f). The linearity correlative index R^2 were 0.992 and 0.953, and linearity ranges were 0.06–3 ng/mL and 0.03–2 ng/mL for PG1 and PG2 detection, respectively. The results of PGs antigens standard samples by ELISA, the LOD were observed with Fig. 1(e)(f) were 2.5 ng/mL of PG1 and 0.676 ng/mL of PG2. Its linearity reach R^2 were 0.998 and 0.951 similar to the detected results on PE-Lamb. The analytical performances of PE-Lamb are comparable or even better than those of the recently reported methods (Table 3).

The PE-Lamb was tested by PGs in healthy human serum. Different concentrations of healthy human serum diluted antigens were detected according to the following: 0 pg/mL PG1 + 0 pg/mL PG2; 250 pg/mL PG1 + 250 pg/mL PG2; 1000 pg/mL PG1 + 250 pg/mL PG2.

The central frequency response on different cavities as shown in Fig. 1(a) (b). The diluted serum produced 40 Hz and 61 Hz frequency shift at anti-PG1 modified cavity and anti-PG2 modified cavity respectively. The frequency shift were 1161.93 Hz in 250 pg/mL and 250 pg/mL test with serum and 2013.30 Hz in 1000 pg/mL and 250 pg/mL test with serum on anti-PG1 modified cavity. The frequency shift were 1548.80 Hz for 250 pg/mL PG1 mixed with 250 pg/mL PG2 in human serum detected on anti-PG2 modified cavity. 1680.83 Hz for 1000 pg/mL PG1 mixed with 250 pg/mL PG2 in human serum on anti-PG1 modified cavity, so just 8.52% frequency influence for 750 pg/mL PG1 in PG2 test. The sensitivity of PE-Lamb is significant and the results in accordance with the test results of the standard sample in the PBS buffer solution as shown in Fig. 1(e) (f).

3.3. Control experiment on PE-Lamb

All the experimental procedures were consistent with the Section 2.4. Different concentrations of standard PG1 and PG2 antigens mixed in PBS buffer were detected according to different concentrations of healthy human serum diluted antigens in Section 3.2.

The frequency shift were 3.42 Hz and −4.54 Hz in 0 pg/mL PG1 + 0 pg/mL PG2 in two detection cavities respectively. The frequency shift were −3.32 Hz and −6.67 for 250 pg/mL PG1 mixed with 250 pg/mL PG2 in PBS buffer detected by two detection cavities. 4.03 Hz and −2.37 Hz were the frequency shift of the 1000 pg/mL PG1 + 250 pg/mL PG2 in two detection cavities respectively. The central frequency response curves could not be differentiated and the frequency shift of the experiments were almost all 0 Hz on each cavities with the 2000 Hz of the span, which showed the PE-Lamb has a good performance in functionality.

3.4. Specificity and interference experiments on PE-Lamb

250 pg/mL, 500 pg/mL and 1000 pg/mL single standard PG1 and PG2 in PBS buffer were detected by PE-Lamb, and all the experimental procedures were consistent with the Section 2.4.

The different concentrations of PG1 antigens were led to 1203.22 Hz, 1602.68 Hz and 1811.93 Hz of the frequency shift on anti-PG1 modified cavity, and just 33.13 Hz, 47.96 Hz and 38.13 Hz on anti-PG2 modified cavity simultaneously. The frequency shift were 42.11 Hz, 29.73 Hz and 50.37 Hz in different concentrations of PG2 antigens on anti-PG1 modified cavity, and 1177.51 Hz, 1503.39 Hz and 1913.42 Hz on anti-PG2 modified cavity simultaneously. Fig. 2 showed the results of the specificity of the PE-Lamb.

4. Conclusion

In this study, we have reported the first time a successful development of PE-Lamb biosensor based microfluidic device which have shown high sensitivity in a label-free detection of PGs for early screening of gastric cancer. PE-Lamb reduced measurements time with significantly improved results compared with traditional ELISA. The

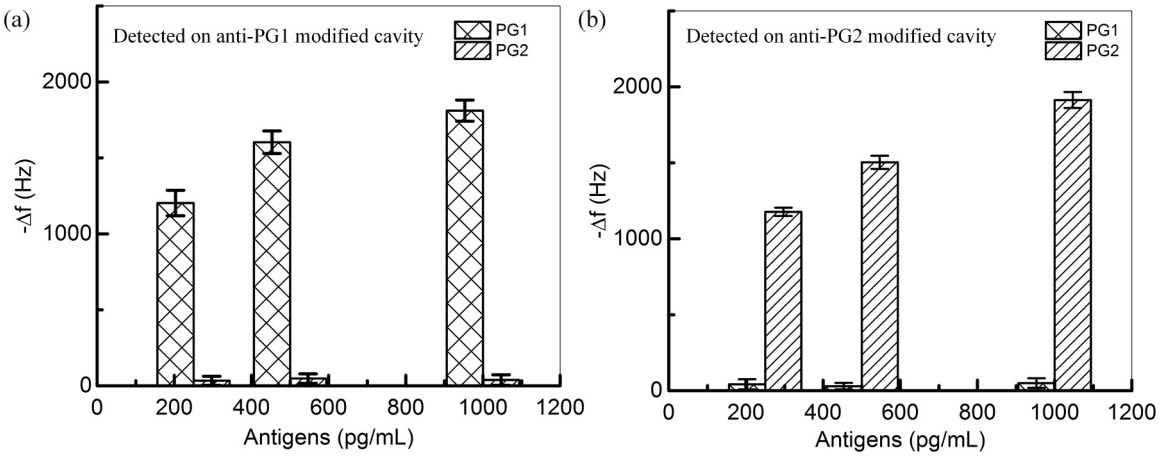


Fig. 2. The results of the single PG1 or PG2 antigens detection on PE-Lamb. (a) The results of different concentrations of PG1 and PG2 antigens detection on anti-PG1 modified cavity; (b) The results of different concentrations of PG1 and PG2 antigens detection on anti-PG2 modified cavity.

PE-Lamb's LOD could reach 60 pg/mL of PG1 diluted by PBS buffer and 30 pg/mL of PG2 diluted by PBS buffer in less than 40 min in drying state. The R^2 of PE-Lamb methods were 0.992 and 0.953 for PG1 and PG2 detection individually, which demonstrate advantageous to the ELISA in LOD and linearity. The liner relationships obtained in the range 60–3000 pg/mL of PG1 and 30–2000 pg/mL of PG2, these linear range were greater than some reported methods, such as optical methods (Cho et al., 2016; Fan et al., 2017; Huang et al., 1987; Jikihara et al., 1992; Wu et al., 2017; Xie et al., 2015; Yasukawa et al., 2007) and electrochemistry methods (Xie et al., 2015) for PGs detection. The lower detection limit may achieve early detection of gastric cancer. Preliminary experiments in which human serum were used as background and diluent, the PE-Lamb was showed high sensitivity and specificity.

The PE-Lamb is capable of small sample volume (at least 20 μL), multiple indicators can be detected with the low LOD, and easy to operate, simultaneously. Small physical size and low cost to be achieved, which will be very suitable for development of a PGs point of care testing (POCT) microsystem, and it is suitable for screening and diagnosis of large-scale early gastric cancer.

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