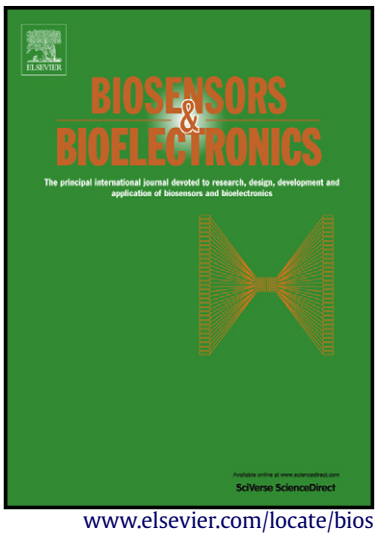

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Label-free and high-sensitive detection of human breast cancer cells by aptamer-based leaky surface acoustic wave biosensor array

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Abstract

A label-free and high-sensitive sensing technology for tumor cell recognition and detection was developed based on a novel 2×3 model of leaky surface acoustic wave (LSAW) aptasensor array. In this methodology, every resonator crystal unit of the LSAW aptasensor array had an individual oscillator circuit to work without mutual interference, and could oscillate independently with the phase shift stability of ± 0.15 degree in air phase and ± 0.3 degree in liquid phase. The aptamer was firstly assembled to the gold electrode surface of 100MHz $LiTaO_3$ piezoelectric crystal, which could effectively captured target cells (MCF-7 cells) based on the specific interaction between aptamer and the overexpression of MUC1 protein on tumor cell surface. The aptamer-cell complexes increased the mass loading of LSAW aptasensor and led to phase shifts of LSAW. The plot of phase shift against the logarithm of concentration of MCF-7 cells was linear over the range from 1×10^2 cells mL^{-1} to 1×10^7 cells mL^{-1} with a correlation coefficient of 0.994. The detection limit as low as 32 cells mL^{-1} was achieved for MCF-7 cells. The LSAW aptasensor also exhibited excellent specificity and stability. In addition, this aptasensor could be regenerated for ten times without irreversible loss of activity. Therefore, the LSAW aptasensor may offer a promising approach for tumor cell detection and have great potential in clinical applications.

Keywords: Leaky surface acoustic wave; Aptasensor; Array; MCF-7 cells

1. Introduction

Breast cancer is the second leading cause of cancer death among women, and more than 90% of cancer deaths attribute to the results of tumor metastasis (Siegel et al. 2012). Monitoring and detecting the existence of distant metastasis is vital importance for selecting the suitable treatment and reducing the rate of tumor recurrence (Geiger and Peeper 2009; Liberko et al. 2013). Unfortunately, conventional histological method, radiologic evaluation, and biomarker could not provide enough information of metastasis to evaluate the clinical outcome and antitumor treatment efficacy as early as possible (Pantel and Alix-Panabieres 2010). Therefore, the development of new feasible diagnostic methods to accurately, rapidly, and simply identify the tumor metastasis has been of great importance.

Circulating tumor cells (CTCs) in peripheral blood are considered as a valuable biomarker for tumor metastasis and may be the origin of incurable metastatic disease (Lianidou and Markou 2011). CTCs have been proven to significantly associate with the overall and progression-free survival in metastatic tumor (Hayes and Smerage 2008; Pantel et al. 2008). Moreover, CTCs are also termed as a real-time “liquid biopsy” due to relative ease of obtaining from peripheral blood sample (Nadal et al. 2013). Currently, CTCs could be specifically determined by combining enrichment process with detection process. Two strategies have been established to detect the CTCs, which are known as immunophenotyping methods (CellSearch, EPISPOT, CTC-chip, etc.) and amplification of malignant cell mutations by PCR (Banys et al. 2013; Criscitiello et al. 2010). However, these methods suffer from false-positive or

false-negative results, time consuming, lack of efficiency, and requiring labeled molecule, all of which limit their clinical applications. Meanwhile, different methods indicate significant differences in CTCs detection rates (23%-100%). Thus, a label-free and high-sensitive method for CTCs detection is urgently needed for early diagnosis of tumor metastasis.

Sensor science and technology emerged as substantially progressive in the past few years. Surface acoustic wave (SAW) biosensors have been developed as powerful and promising biosensor systems for application in a variety of fields, such as biochemistry, clinical analysis, and environmental monitoring (Gronewold 2007; Voiculescu and Nordin 2012). The advantages of SAW biosensors are extremely high sensitivity, label-free detection, and good reproducibility (Afzal et al. 2013). Recently, leaky surface acoustic wave (LSAW) biosensor, as one kind of SAW biosensors, has been developed with the progress of microelectronic and acoustic technique (Belovickis et al. 2012; Inoue et al. 2007). The LSAW biosensor excites a LSAW mode based on a special orientation of piezoelectric crystal, such as the 36º rotated Y-cut X propagation lithium tantalate ($LiTaO_3$) piezoelectric single crystal. In this case of crystal structure, the LSAW propagates along the surface of a medium and hence leads to high energy density along the surface. In addition, the LSAW generally has a higher phase velocity, lower propagation loss, and larger electromechanical coupling factor than that of the conventional Rayleigh waves (Meng et al. 2011; Rocha-Gaso et al. 2009). Compared with our constructed quartz crystal microbalances (Chen et al. 2005; Luo et al. 2006), LSAW biosensor allows to achieve higher

operation frequencies (up to 100MHz) with the same photolithography-defined transducer dimensions. Therefore, the LSAW biosensor is potentially sensitive to any change on the surface, such as mass loading and conductivity changes.

In our previous study, we have set up a LSAW biosensor platform and successfully detected human papilloma virus and Japanese encephalitis virus (Wang et al. 2009; Xu et al. 2012). To our knowledge, there are no reports on detecting CTCs by LSAW biosensor. In the present work, we constructed a novel aptamer-based LSAW biosensor (LSAW aptasensor) array for label-free and high-sensitive detection of CTCs. The 2×3 model of LSAW aptasensor array was designed to improve the efficiency of CTCs detection. Michigan cancer foundation-7 (MCF-7) human breast cancer cell was used as the model target to test our new label-free sensing technique. Subsequently, the main experimental condition of LSAW aptasensor was discussed in detail. Besides, the LSAW aptasensor was proven to linearly detect MCF-7 cells in a wide range from 1×10^2 cells mL^{-1} to 1×10^7 cells mL^{-1}, and exhibited a detection limit of 32 cells mL^{-1}. This method could also distinguish MCF-7 cells from other kinds of cells, such as Romas cells and K562 cells. Therefore, the LSAW aptasensor array may have great potential in future application.

2. Materials and methods

2.1 Reagents

Thiolated DNA sequence (HPLC purified) was manufactured by Shanghai Bioengineering Company (Shanghai, China). The base sequence of MUC1 aptamer was 5'-HS-GCAGTTGATCCTTTGGATACCCTGG-3'. The random sequence was

5'-HS-CACGACGTTGTAAAACGACGGCCAG-3'. Tween-20, bovine serum albumin (BSA, 96%–99%), 6-mercapto-1-hexanol (MCH), and tris (2-carboxyethyl) phosphine hydrochloride (TCEP) were obtained from Sigma-Aldrich (St Louis, MO, USA). Piranha solution (1:3 mixture of 30% hydrogen peroxide and 98% sulfuric acid) and phosphate buffered saline (PBS) buffer were prepared in the laboratory. All chemical reagents used were of analytical reagent grade. The ultrapure water was used with a specific resistance over 18.0 MΩ cm (Millipore Purification Pack).

2.2 Fabrication of LSAW biosensor and assembly of 2×3 model microarray

A 100 MHz LSAW aptasensor was designed as two-port resonator. The device was fabricated on single side polished 36°YX-lithium tantalite ($LiTaO_3$). The $LiTaO_3$ crystal was initially rinsed with acetone, isopropyl alcohol, and ultrapure water in order, and then dried with nitrogen gas. The interdigital transducer (IDT) consisted of 100 finger pairs at the input port and output port, and was patterned on the surface of $LiTaO_3$ crystal by metal evaporation, reactive ion etching (RIE), and ion sputtering techniques. The LSAW propagation was excited and detected by the IDT. The length of IDT was 17.0 μm with an aperture of 4.0 mm. Subsequently, the two sets of reflectors were deposited by e-beam evaporation and etched in the outside of IDTs. The reflectors were used to improve the signal noise ratio and sensitivity of sensor by increasing the acoustic wave propagating path. The metal layers were deposited by the vacuum ion sputtering technique. They comprised of a thin chromium layer followed by a gold layer. The detailed configuration of LSAW aptasensor was shown in Fig. 1A. The 2×3 model of LSAW aptasensor array (including five detection sensors and one

reference sensor) was shown in Fig. 1B. The LSAW aptasensor and array were co-designed and developed by our laboratory and the 26th Research Institute of the China Electronics Technology Group.

2.3 Sensor system and other instruments

The structure of LSAW biosensor system was shown in Fig. 1C. The oscillating signals from LSAW biosensor automatically input into a NI-PXI data collection system (National Instruments, Austin, TX, USA). The data collection, storage, and processing was conducted by using the self-developed biosensor system monitor software (BSMS 2.0). The phase data were displayed on the main display screen and could be directly read by a computer connected to the NI-PXI data collection system. The real-time monitoring signals for LSAW aptasensor detection were shown in Fig S1 (Supplementary materials).

2.4 Cell culture and preparation

Human breast cancer cell lines MCF-7 as target cells, Romas cells and K562 cells as control cells were obtained from ATCC (Manassas, VA, USA). All cell culture mediums and supplements were purchased from Gibco BRL (Carlsbad, CA, USA). MCF-7 cells were cultured in low-glucose Dulbecco's modified Eagle's medium (DMEM), while Romas cells and K562 cells were grown in RPMI 1640 medium. All mediums were supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. The cells were incubated in a humidified atmosphere with 5% CO_2 at 37°C. After growing to 90% confluence, the MCF-7 cells were harvested by trypsinization. Subsequently, the cells were firstly centrifuged at 1500 rpm for 5min,

and then incubated in 100 μL of PBS buffer containing 0.5% Tween-20 and 1% BSA for 30min. After pretreating, the cells were centrifuged at 1500 rpm for 5min and resuspended in PBS buffer (pH 7.4) containing 1mM Ca^{2+} and 1mM Mg^{2+}. The cell density was counted by the hemocytometer prior to each experiment. The cell suspension was then diluted to the desired concentration.

2.5 Immobilization procedures

After sputter coating with gold, the gold membrane of LSAW aptasensor was mechanically cleaned with ultrasound. Then, the gold electrode was immersed in piranha solution (30% H_2O_2: 98% H_2SO_4 = 1: 3) for 10 min, rinsed three times with ultrapure water, and finally dried with nitrogen gas. Subsequently, the gold electrode surface of detection sensor was soaked in MUC1 aptamer solution (10 mM Tris-HCl, 1 mM EDTA, 1.0 M NaCl, and 1 mM TCEP) for 24h. The corresponding reference sensor was immobilized with random sequence. To block unoccupied binding site, an aqueous solution of MCH (1mM) was laid on the electrode surface and incubated 2 h. After being cleaned and dried, the aptasensor was ready for the later experiment.

2.6 Detection procedures

The detection sensor and reference sensor were filed with 36 μL of PBS buffer (pH 7.4) containing 1mM Ca^{2+} and 1mM Mg^{2+}, respectively. The phase (P_0) of LSAW aptasensor was monitored until a steady baseline. Then different concentrations of cell solution were added to the detection and reference sensor, respectively. The aptasensor was maintained at 37°C for reaction. When the resonance phase reached a stable value, steady-state phase was taken as P_1. The phase shifts were calculated by

equation $PS = P_1 - P_0$. The phase shifts of detection sensor (PS_1) and reference sensor (PS_2) were determined, and the relative phase shift attributed to aptamer-cell reaction was calculated by equation $\Delta\,\text{Phase} = PS_1 - PS_2$. The whole detection procedure was monitored, and the real-time phase shift was automatically recorded and displayed via BSMS 2.0 software.

2.7 Specificity procedures

To assess the specificity, LSAW aptasensor was performed to detect the Romas cells and K562 cells. After cleaning the LSAW aptasensor, MUC1 aptamer was immobilized on the gold electrode surface of detection sensor. The corresponding reference sensor was immobilized with random sequence. Subsequently, the Romas cells and K562 cells were respectively added into LSAW aptasensor to explore the specificity. The Romas cells and K562 cells were resuspended in PBS buffer (pH 7.4) containing 1mM Ca^{2+} and 1mM Mg^{2+} in a concentration of 1×10^7 cells mL^{-1}.

2.8 Regeneration procedures

The EDTA solution (30 mM) was used as the regeneration reagents. After each measurement, the gold electrode surface of LSAW aptasensor was immersed in regeneration solution to denature the aptamer and remove the CTCs. The obtained surfaces were then rinsed with PBS buffer 3 times. After washing with ultrapure water, the aptasensor was once again responded to the MCF-7 cells in a concentration of 1×10^5 cells mL^{-1}. To test whether the activity of LSAW aptasensor was irreversible loss by the regeneration process, this procedure was repeated ten times with one LSAW aptasensor and one sample.

2.9 Data analyses

Statistical analysis was performed using SPSS 13.0 software package (SPSS Inc, Chicago, IL, USA) and GraphPad Prism 5.0 software (GraphPad Software Inc, LaJolla, CA, USA). The ΔPhase data were expressed as mean ± standard deviation of at least three independent experiments. Multiple-group statistical analyses were performed by One-way of variance followed by least significant difference post hoc testing. A p value of less than 0.05 was considered statistically significant.

3. Results and discussion

3.1 Oscillation property of LSAW biosensor array

The two-port resonator LSAW aptasensor was fabricated using 36°YX $LiTaO_3$ as piezoelectric substrates. The LSAW was excited based on the inverse piezoelectric effect of $LiTaO_3$ crystal. The displacement of LSAW was along the surface of a medium. Hence, the wave had minimal damping when the surface of sensor was in contact with liquid environments. The characteristic parameters of LSAW biosensor were shown in the supplementary materials. In addition, the resonator model of LSAW aptasensor could oscillate stably in liquid phase. In our experiment, the results indicated that LSAW aptasensor has powerful oscillation ability and stable phase shift with the stability of ± 0.15 degree in air phase and ± 0.3 degree in PBS buffer for more than 40 min (data not shown). Moreover, the 2×3 model of LSAW aptasensor included five detection sensors (immobilized by MUC1 aptamer) and one reference sensor (immobilized by random sequence). The reference sensor was used to normalize the responses of detection sensor and reduce the interference from

non-specific adsorption, which would also increase the oscillation stability of sensor. Every oscillator of the array was independently designed from each other and driven by the individual oscillator circuits, therefore each aptasensor could work well without mutual interference. Meanwhile, compared with previous single-channel sensor (Wang et al. 2009; Xu et al. 2012), the 2×3 model of array could analyze 5 samples simultaneously and reduce the time required for processing of a number of samples. Especially, the 2×3 model of array had the advantage of convenient assembly according to the request of clinical diagnosis.

3.2 The principle of the LSAW aptasensor

Aptamers are selected through systematic evolution of ligands by exponential enrichment (SELEX). The aptamers are capable of binding with specific molecules because of their well-defined secondary structure (Gong et al. 2012). In comparison to antibody as capture molecule, the aptamer has tremendous potential for its low molecular weight, easy synthesis and modification, denser receptor layer, and long-term stability (Fu et al. 2013; Liu et al. 2009). All these properties collectively make aptamer to be an excellent alterative as molecular probes for biomedical studies. Up to now, various aptasensors have been constructed for detecting a broad range of analytes, including small molecules, bacteria, viruses, and even cells (Hong et al. 2012). Aptasensors based on fluorescence and electrochemistry assay have been developed, and attracted particular attention in CTCs detection (Feng et al. 2011; Zheng et al. 2009). However, their manipulation procedures are relatively time-consuming and require labeled molecule. The LSAW aptasensor was used for

MCF-7 cells sensing, it not only had the advantages of LSAW biosensor, such as high sensitivity, label-free detection, and rapid response, but also combined with the high specificity and affinity of aptamers.

To illustrate the strategy for high-sensitive detection of CTCs, the principle of LSAW aptasensor was demonstrated in Fig.2. The system included mercapto modified aptamer sequence and random sequence. The mercapto modified aptamer was firstly immobilized on detection sensor, and random sequence was immobilized on reference sensor. Subsequently, the MCF-7 cells were captured based on the specific interaction between aptamers and mucin 1 proteins (MUC1) on the cell surface. The MUC1 is the most common tumor marker for monitoring metastatic breast cancer. As cell surface-associated glycoprotein, the MUC1 is associated with a poor prognosis in breast cancer, and elevated level of MUC1 has been considered to be a significant indicator in the diagnosis of breast cancer (Shen et al. 2008). Therefore, the phase shift of LSAW aptasensor was only observed in detection sensor, and the MCF-7 cells could be detected accurately and specifically.

3.3 Optimization of immobilized aptamer concentrations

The LSAW aptasensor utilizing aptamers as recognition elements detected CTCs. Thus, the construction of sensing layer was the most important stage in design of LSAW aptasensor. It is widely accepted that the surface density of aptamer plays a crucial role in construction of sensing layer. In order to evaluate the effect of aptamer concentration on ΔPhase, a series of aptamer concentrations from 0.5 μM to 8.0 μM were immobilized on the surface of detection sensor. The reference sensor was filled

with corresponding concentrations of random sequence. Then, the final concentration of 1×10^5 cells mL^{-1} was added into detection sensor and reference sensor, respectively. The LSAW aptasensor was maintained at 37°C for reaction. The results indicated the ΔPhase increased with aptamer concentrations ranging from 0.5 μM to 2.0 μM and significantly decreased from 2.0 μM to 8.0 μM (Fig.3). The LSAW aptasensor displayed an optimum sensitivity of the response at aptamer concentration of 2.0 μM. The coefficients of variation (CVs) were shown in Table.S1. The spatial arrangement and surface density of aptamer may explain the effect of aptamer concentration on Δ Phase. When the aptamer concentration was below the threshold, the aptamer could efficiently capture CTCs without overcoming any steric hindrance. With the buildup of aptamer, the denser sensing layers were generated on the surface of sensor. In this case, the aptamer was sterically repelled by neighboring aptamer, which provided a limited space for the interaction between the secondary structure of aptamer and MCF-7 cells (Luo et al. 2006). In addition to spatial restriction, the electrostatic repulsion between aptamers may also partially involve in the effect of aptamer concentration on ΔPhase.

3.4 The analytical performance of LSAW aptasensor

Under the optimized test conditions, the quantitative behavior of LSAW aptasensor was assessed by detecting different concentrations of MCF-7 cells (10 cells mL^{-1}, 1×10^2 cells mL^{-1}, 1×10^3 cells mL^{-1}, 1×10^4 cells mL^{-1}, 1×10^5 cells mL^{-1}, 1×10^6 cells mL^{-1}, 1×10^7 cells mL^{-1}). The real-time detection signals of different concentrations of MCF-7 cells were shown in Fig.4A. The ΔPhase dynamically increased with the

rising concentration of MCF-7 cells from 10 cells mL^{-1} to 1×10^7 cells mL^{-1}. The results indicated that the number of aptamer-cell complexes was increased with the rising concentration of MCF-7 cells. The average ΔPhase of LSAW aptasensor was linear to the logarithm of concentration of MCF-7 cells in the range from 1×10^2 cells mL^{-1} to 1×10^7 cells mL^{-1} with a correlation coefficient of 0.994 (Fig. 4B). The linear regression equation was calculated as ΔPhase = 0.465 lg C-0.112, where C was the concentration of MCF-7 cells (cells mL^{-1}). Compared with previous methods of CTCs detection (Hun et al. 2011; Li et al. 2010), the LSAW aptasensor presented a wider detection range with MCF-7 cells measurements. The threshold of detection limit was set at 3-fold standard deviation (SD) above blank. Therefore, the detection limit was determined as 32 cells mL^{-1}. The CVs of ΔPhase obtained from different concentrations of MCF-7 cells were shown in Table S2.

Detection limit is an important parameter for biosensor. Our results revealed that the detection limit of LSAW aptasensor was much lower than those reported label-free methods, such as peptide nanotube-folic acid modified graphene electrode (8×10^3 cells mL^{-1}) (Pan et al. 2010), aptamer-conjugated near-infrared fluorescent nanoparticles (4×10^3 cells mL^{-1}) (Deng et al. 2010), and electrochemical cytosensor array (794 cells mL^{-1}) (Feng et al. 2011). Meanwhile, the detection limit of LSAW aptasensor was comparable to the enzyme assisted amplified aptamer-based assays (Zhang et al. 2011; Zhao et al. 2013). In addition, The MCF-7 cells were reproducibly measured in all concentrations with coefficients of variation less than 10%, which indicated an acceptable reproducibility.

3.5 Specificity and stability of LSAW aptasensor

The specificity is critical importance in cancer detection. The non-target cells may cause high signal backgrounds that affect the specificity of aptasensor. Thus, we performed experiments to estimate the specificity of LSAW aptasensor by detecting other two kinds of cells, Romas cells and K562 cells. The MCF-7 cells, Romas cells, and K562 cells were respectively detected under the same procedure as depicted in experimental section. Fig. 5 showed the ΔPhase obtained in the presence of 1×10^7 cells mL^{-1} MCF-7 cells, Roams cells, and K562 cells. A well-defined ΔPhase could be observed in the presence of MCF-7 cells, whereas the ΔPhase of Roams cells or K562 cells was approximately the same as blank solution. The results revealed the LSAW aptasensor had an excellent capability in discriminating MCF-7 cells from other two kinds of cells. The high specificity of LSAW aptasensor may ascribe to the affinity of MUC1 aptamer to MCF-7 cells.

Stability of aptasensor is another important aspect for the practical application. The long-term stability of LSAW aptasensor was investigated in this study. The sensor was dried and stored at 4°C。After 4 weeks, the ΔPhase of LSAW aptasensor were about 90% compared with its initial response. This indicated the storage stability of LSAW aptasensor was acceptable.

3.6 Regeneration of LSAW aptasensor

The aptasensor not only is sensitive and specific to target cells, but also is reusable. However, the regeneration is still a challenge for most existing biosensors (Du et al. 2008). Compared with antibody, the aptamer is less vulnerable to degradation and has

much longer shelf life (Yan et al. 2013). In addition, the selection of aptamer must undergo denaturation and refolding steps during each round of SELEX, which has to some extent increased the regeneration. All of these properties suggested that the aptamer-based biosensor might be regenerated. In order to determine the regeneration of LSAW aptasensor, a mild regeneration reagent EDTA (30mM) was used to dissociate the adsorptive CTCs completely without irreversible loss of its activity. After five regenerating cycles, the sensing activity of LSAW aptasensor has possessed 90.9% of the original, and after ten regenerating cycles, the sensing activity has possessed 72.7% of the original (Fig. 6). Value of CV was 9.79%, i.e., less than 10%, which indicated a feasible regeneration of LSAW aptasensor. However, the regenerating cycles of LSAW aptasensor was less than five times in the practical application, so as to ensure the accuracy of CTCs detection. The EDTA could be used to remove the CTCs and regenerate aptasensor. This is because the aptamers need bivalent metal ions to form the secondary structure and unfold in the presence of EDTA. The aptamer could rapidly refold when EDTA is withdrawn and replaced by the solution containing Mg^{2+} (Liss et al. 2002).

Besides of the aforementioned features, the LSAW aptasensor also allowed rapid detection and easy handling. The total detection time was within 40 min, which was much shorter in comparison to that of the conventional methods such as immunophenotyping methods and PCR techniques. Furthermore, the LSAW aptasensor only needed a single aptamer and a random sequence, the label-free detection of MCF-7 cells could be accomplished with one step. The LSAW aptasensor

not only decreased the fluorescence interference in detection, but also was relatively easy to operate and low detection cost. Consequently, the LSAW aptasensor may provide a convenient strategy for monitoring CTCs.

4. Conclusions

In this study, we reported a label-free and high-sensitive approach for detection of MCF-7 cells by a novel 2×3 model of LSAW aptasensor array. This technique combined the sensitivity of LSAW biosensor with the specificity of aptamer recognition, and provided a highly versatile platform for the detection of CTCs. The LSAW aptasensor demonstrated a linear relationship with the concentration of MCF-7 cells ranging from 1×10^2 cells mL^{-1} to 1×10^7 cells mL^{-1}, and a detection limit of 32 cells mL^{-1} was achieved. In addition, the design and format of micro-array aptasensor significantly increased the fluxes of detection. These features, as well as its regeneration and stability, made LSAW aptasensor a promising alternative to conventional cancer detection methods. Therefore, the self-designed LSAW aptasensor array may provide more effective and accurate prognosis evaluation and therapeutic monitoring for cancer patients. In the future, this aptasensor may be expanded as a potentially generalized method to detect more kinds of CTCs by altering the immobilized aptamers according to the clinic demands. However, more detailed work should be done before the clinical application.

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Highlights

- A novel 2×3 model of LSAW aptasensor array for MCF-7 cells detection was constructed.
- The phase shift was linear to the logarithm of MCF-7 cells concentration.
- The LSAW aptasensor exhibited a feasible regeneration and excellent specificity.

Figures

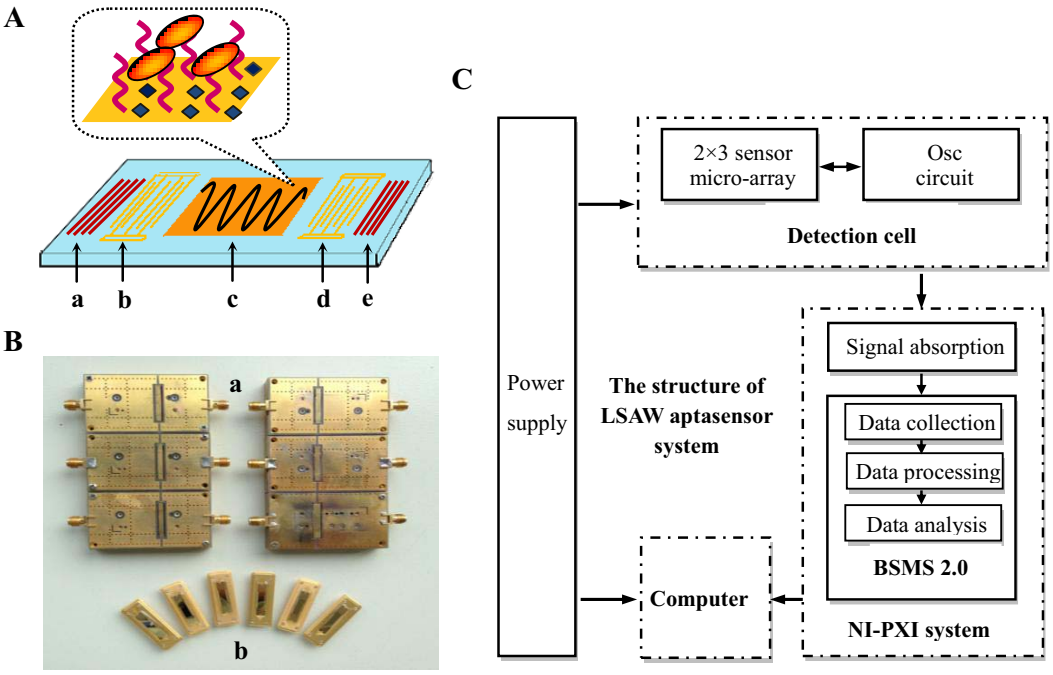

Fig.1. Characteristic of LSAW aptasensor array. (A) The structure of LSAW aptasensor. (a) input reflectors, (b) input IDT, (c) reaction area, (d) output IDT, (e) output reflectors. (B) Schematic diagram of 2×3 model of LSAW aptasensor array. (a) 2×3 model of sensor array, (b) photograph of a LSAW aptasensor. (C) The detecting system of LSAW aptasensor.

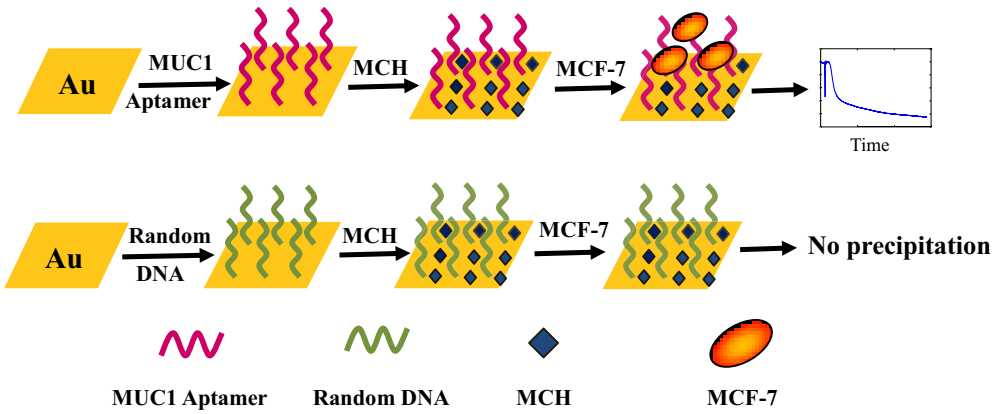

Fig.2. Schematic illustration of the strategy for detection of MCF-7 human breast cancer cells based on LSAW aptasensor.

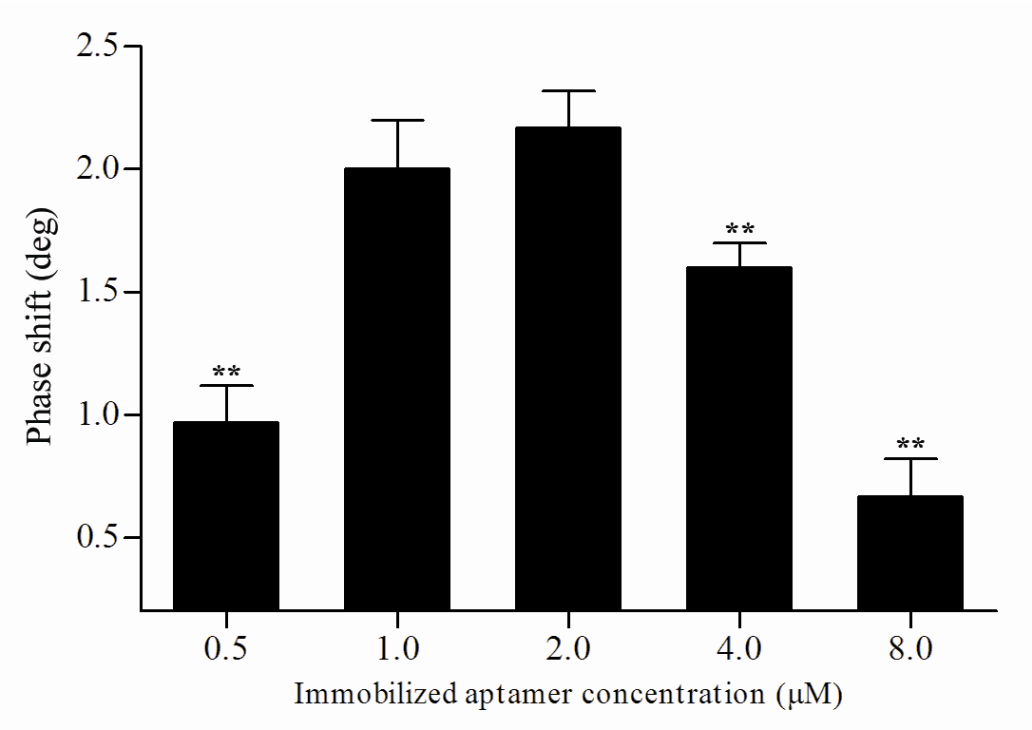

Fig.3. Graph of Δ Phase with various immobilized MUC1 aptamer concentrations, *vs*. 2.0 μM group. A concentration (1×10^5 cells mL^{-1}) of MCF-7 cells was added to determine the optimal aptamer concentration. ** $p<0.01$.

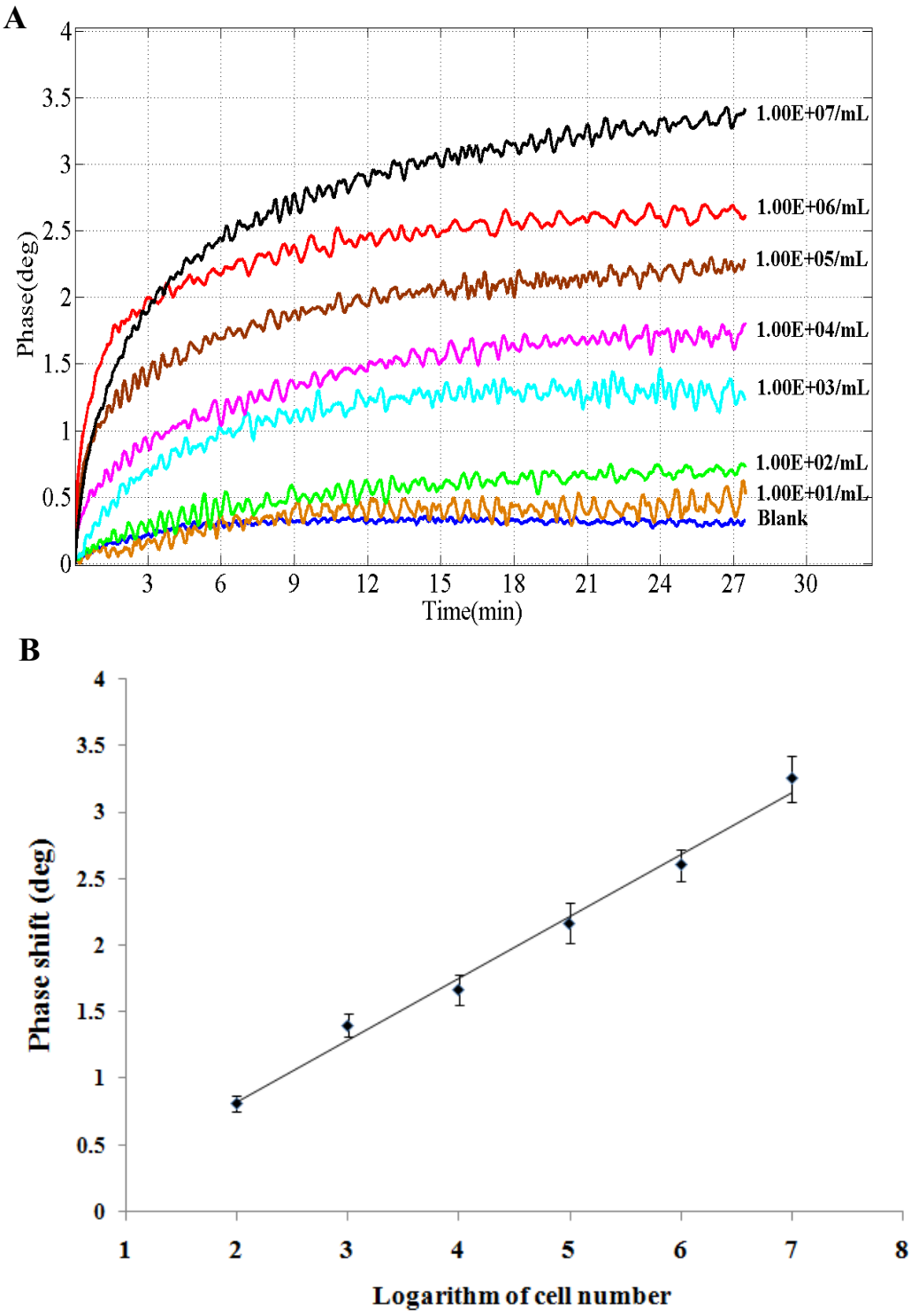

Fig.4. (A) The real-time monitoring signals of aptamer-cell complexes at different MCF-7 cells concentrations with LSAW aptasensor. (B) The standard curve for quantitative detection of MCF-7 cells with LSAW aptasensor. The ΔPhase on the

y-axis was linear relationship with the increasing concentrations of MCF-7 cells on the x-axis. Each data point represented an average of least three times measurements ± standard deviation.

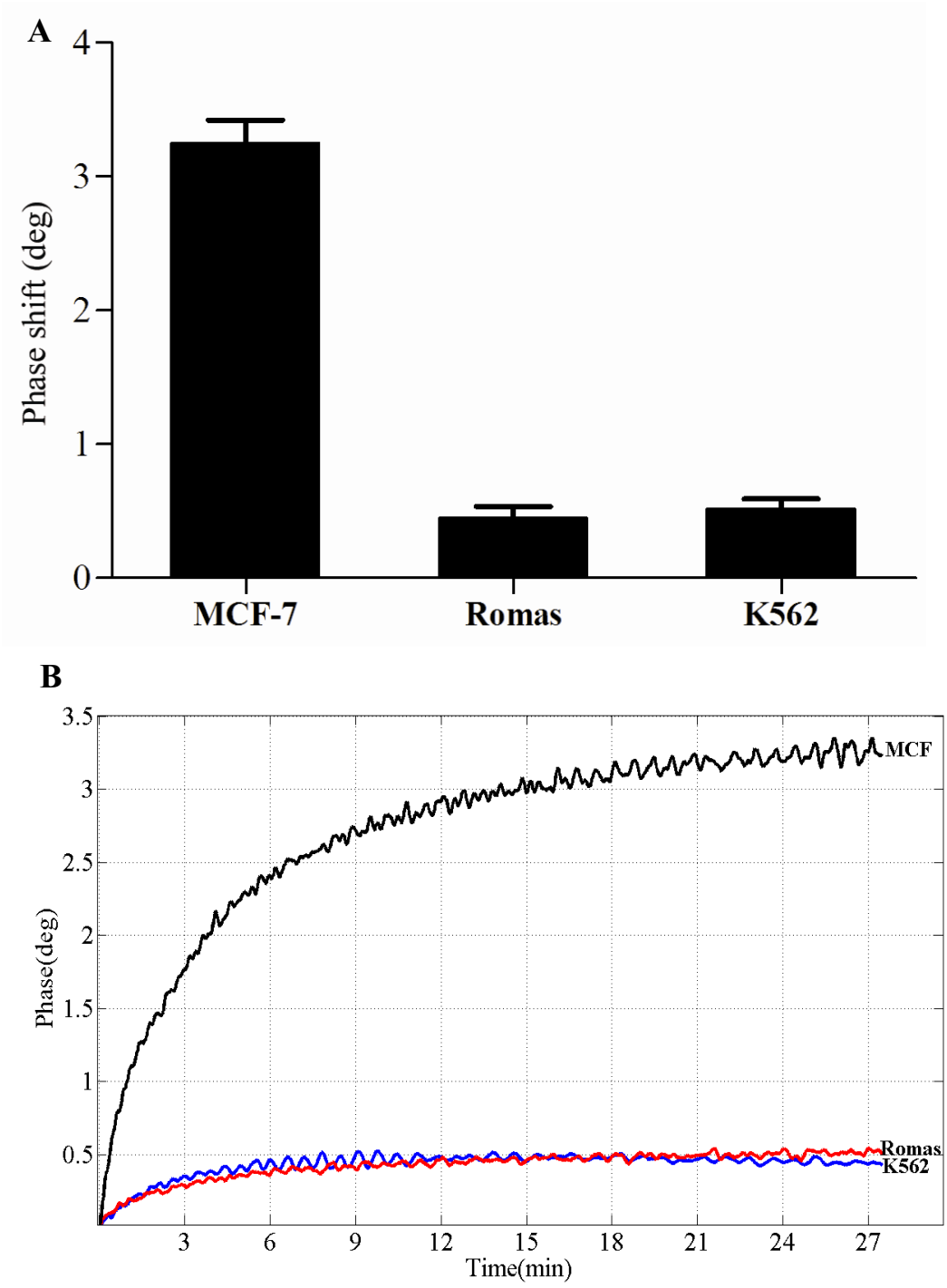

Fig.5. (A) The relationship between the ΔPhase and different cell types. (B) The real-time detection signals of different cell types. The MCF-7 cells, Romas cells, and K562 cells were analyzed at a concentration of 1×10^{7} cells mL^{-1}. Three replicates per cell type were measured.

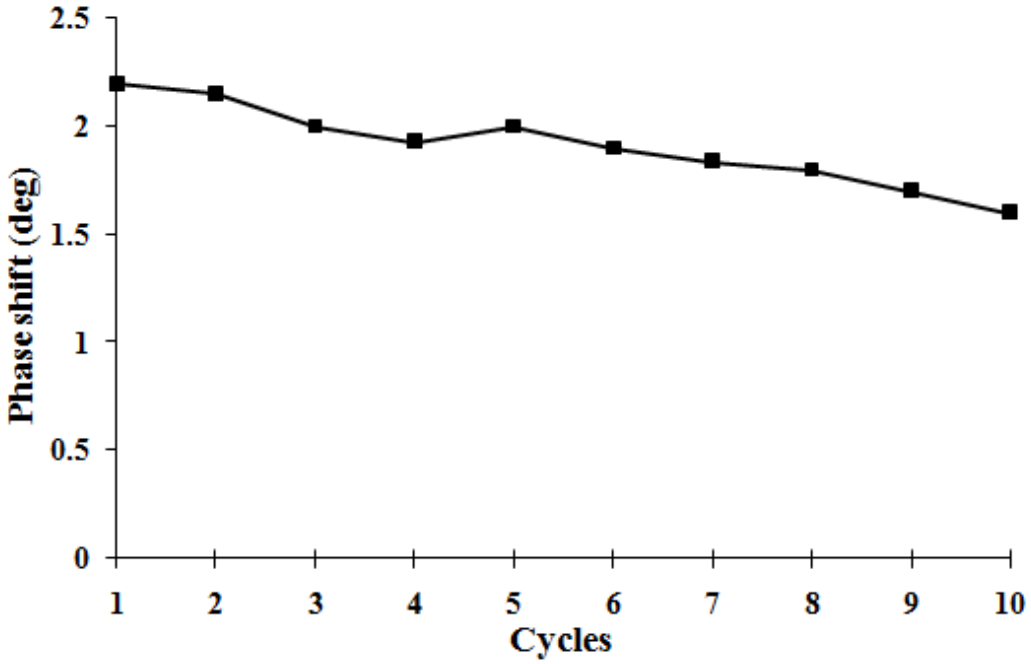

Fig.6. Relationship between responses activity changes of LSAW aptasensor array and number of regeneration cycles.