Biosensors and Bioelectronics 60 (2014) 318–324

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

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

Kai Chang [a], Yan Pi [b], Weiping Lu [a], Feng Wang [a], Feng Pan [a], Fake Li [a], Shuangrong Jia [a], Jianfeng Shi [c], Shaoli Deng [a,*], Ming Chen [a,**]

[a] Department of Clinical Laboratory Medicine, Institute of Surgery Research, Daping Hospital, The Third Military Medical University, Chongqing 400042, China
[b] Department of Neurology, Institute of Surgery Research, Daping Hospital, The Third Military Medical University, Chongqing 400042, China
[c] The 26th Research Institute, Chinese Electronic Scientific and Technical Group Company, Chongqing 400060, China

ARTICLE INFO

Article history:
Received 10 January 2014
Received in revised form
26 March 2014
Accepted 17 April 2014
Available online 30 April 2014

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

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 $\pm 0.15^{\circ}$ in air phase and $\pm 0.3^{\circ}$ in liquid phase. The aptamer was firstly assembled to the gold electrode surface of 100 MHz $LiTaO_3$ 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.

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.

* Corresponding author. Tel.: +86 23 68757602; fax: +86 23 68716530.
** Corresponding author. Tel.: +86 23 68757601; fax: +86 23 68716530.
E-mail addresses: dengsl072@yahoo.com.cn (S. Deng), chenming1971@yahoo.com (M. Chen).

http://dx.doi.org/10.1016/j.bios.2014.04.027
0956-5663/

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 36° 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 100 MHz) 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

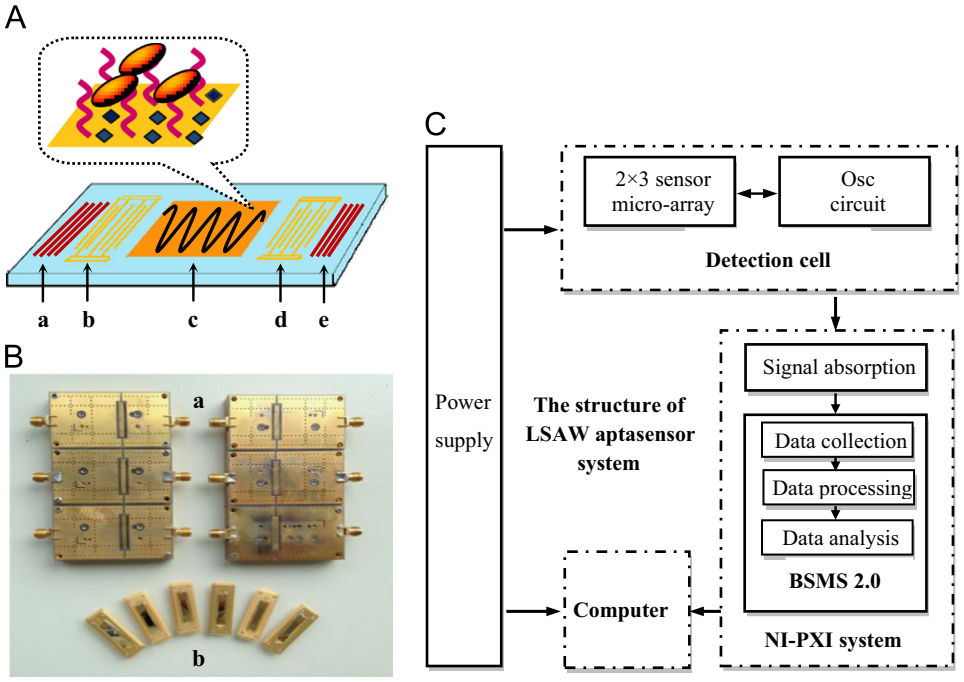

Fig. 1. Characteristic of LSAW aptasensor array. (A) The structure of LSAW aptasensor. (a) input reflectors, (b) input IDT, (c) reaction area, (d) output IDT, and (e) output reflectors. (B) Schematic diagram of 2×3 model of LSAW aptasensor array. (a) 2×3 model of sensor array, (b) photograph of a LSAW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