Biosensors and Bioelectronics 95 (2017) 48–54

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Gold nanoparticle-based low limit of detection Love wave biosensor for carcinoembryonic antigens

Shuangming Li[a,b], Ying Wan[a], Yan Su[a], Chunhai Fan[a], Venkat R. Bhethanabotla[b,*]

[a] School of Mechanical Engineering, Nanjing University of Science and Technology, Nanjing, China
[b] Department of Chemical & Biomedical Engineering, University of South Florida, Tampa, Florida, USA

ARTICLE INFO

Keywords:
Surface acoustic wave
Love wave
Biosensors
CEA
Gold nanoparticles

ABSTRACT

In this work, a Love wave biosensing platform is described for detecting cancer-related biomarker carcinoembryonic antigen (CEA). An ST 90°-X quartz Love wave device with a layer of SiO_2 waveguide was combined with gold nanoparticles (Au NPs) to amplify the mass loading effect of the acoustic wave sensor to achieve a limit of detection of 37 pg/mL. The strategy involves modifying the Au NPs with anti-CEA antibody conjugates to form nanoprobes in a sandwich immunoassay. The unamplified detection limit of the Love wave biosensor is 9.4 ng/mL. This 2–3 order of magnitude reduction in the limit of detection brings the SAW platform into the range useful for clinical diagnosis. Measurement electronics and microfluidics are easily constructed for acoustic wave biosensors, such as the Love wave device described here, allowing for robust platforms for point of care applications for cancer biomarkers in general.

1. Introduction

Accurate quantification of cancer biomarkers is critical for early diagnosis and monitoring. However, these trace protein biomarkers, such as CEA, CA125 and Bcl-2, are present in a very low concentration level in serum or urine of cancer patients (Anderson et al., 2009; Drenberg et al., 2010; Ni et al., 2005). Traditional assay methods such as enzyme-linked immunosorbent assay (ELISA) (Butler, 2000), radioimmunoassay (Kato and Torigoe, 1977), fluorescence immunoassay (Hicks, 1984), electrophoretic immunoassay (Liu et al., 2008) and mass spectrometric immunoassay (Nelson et al., 1995) have some disadvantages, such as requiring labeled markers, long processing times and requiring expensive instruments. Thus, the demand for rapid, operationally simple, ultrasensitive biosensors is increasing, especially those capable of point of care use.

Surface acoustic wave (SAW) biosensors have been used in biological application for many years (Länge et al., 2008). SAW biosensors have the advantages of high sensitivity, small dimension, low cost, and can be used in label-free and real-time monitoring. They therefore have great potential in clinical diagnosis, especially in point of care testing and portable sensing applications (Hur et al., 2005; Länge et al., 2003). The SAW sensor is a piezoelectric mass sensor that can be sensitive to mass loading on the surface, as the loading can influence the propagation of the acoustic wave (Ballantine et al., 1997). Amongst various SAW sensors, Love wave sensor, also named guided shear horizontal SAW sensor, is a favored device for liquid phase applications. This device has a low velocity waveguide layer on a piezoelectric oxide surface in which shear horizontal SAWs are propagated using suitably designed and lithographically patterned interdigital transducers (IDT). The waveguide layer reduces power consumption and increases sensitivity (Gaso et al., 2013). Though the Love wave sensor has a much higher sensitivity than other piezoelectric sensors, such as quartz crystal microbalances (Gaso et al., 2013; Ward and Buttry, 1990; Zhang et al., 2015), its limit of detection for practically useful devices is not low enough to quantify cancer biomarkers at the required pg/mL levels (Anderson et al., 2009).

Nanoparticle-biomolecule hybrid materials have been studied and utilized in biomedical applications recently (Azzazy et al., 2006; Grodzinski et al., 2006). Owing to the unique electronic, photonic, catalytic properties and dimensional similarity, the integration of nanoparticles with biomolecules (e.g. DNA and proteins) leads to novel synergetic functionalized hybrid nanobiomaterials. With the aim of lower limit of detection, various signal amplification strategies based on nanoparticle-biomaterials have been developed. Amongst these, gold nanoparticles (Au NPs) have been studied and used because of numerous advantages, such as good compatibility with biomolecules and high surface-to-volume ratio (Daniel and Astruc, 2004). Au NPs can increase the sensitivity in various ways, such as through the loading of large amounts of electroactive labels in electrochemical immunosensing (Das et al., 2006; Mani et al., 2009), and heavy mass loading in

* Corresponding author.
E-mail address: bhethana@usf.edu (V.R. Bhethanabotla).

http://dx.doi.org/10.1016/j.bios.2017.04.012
Received 23 December 2016; Received in revised form 28 March 2017; Accepted 11 April 2017
Available online 12 April 2017

mass immunosensing (Chu et al., 2012; Ma et al., 2002). For example, Dequaire et al. developed a sensitive electrochemical immunoassay using a colloidal gold label that was indirectly determined by anodic stripping voltammetry (ASV) at a single-use carbon-based screen-printed electrode (SPE). This method was evaluated for a noncompetitive heterogeneous immunoassay of an immunoglobulin G (IgG) and a concentration as low as 3×10^{-12} M was determined (Dequaire et al., 2000). He et al. reported a new approach to ultrasensitive detection of DNA hybridization based on nanoparticle-amplified surface plasmon resonance (SPR). They observed a 2–4 order of magnitude improvement in sensitivity compared with literature values for unamplified scanning (He et al., 2000).

In this work, we present a sensitive carcinoembryonic antigen (CEA) detection strategy based on an Au NP-amplified immunoassay with a Love wave biosensor. CEA is a glycosylphosphatidylinositol cell surface anchored glycoprotein that is a well-known, broad spectrum biomarker related to various cancers (Moertel et al., 1993; Wiggers et al., 1986), and it is also an indicator of disease recurrence (Wanebo et al., 1978). In our previous work, the design and fabrication of various SAW sensors has been presented (Li et al., 2015; Richardson et al., 2015). The orientation of the crystal relative to the propagation direction is an important factor; different cut angles lead to different values for the acoustic velocity, coupling coefficient (K^2), and temperature coefficient of frequency (TCF). Thus, the choice of the crystal orientation becomes a very critical factor. In biosensing applications, the liquid has a large damping effect on the particle movement in the direction normal to the surface, which leads to larger power consumption, therefore, the particle displacement must be polarized in the shear-horizontal direction (Moriizumi et al., 1987). Common choices for piezoelectric substrates to construct Love wave biosensor devices are ST-X quartz and 36° Y-cut lithium tantalate ($LiTaO_3$) (Bender et al., 2000; Schlensog et al., 2004). Besides the advantages of stability, easy integration and low cost, we found that ST quartz SAW devices can combine sample mixing and non-specific binding removal functions, showing potential for future point of care and portable diagnosis applications (Morrill, 2014). Thus, a sensor with a center frequency of about 120 MHz was fabricated in ST-90 ° quartz with a SiO_2 waveguide layer for this work. To achieve a low limit of detection, a sandwich assay with anti-CEA antibody assembled with Au NPs is conceived. The fabricated biosensors were tested with and without the Au NP nanoprobes for CEA quantification at clinically relevant levels of tens of picograms per milliliter.

2. Experimental

2.1. Materials and methods

Carcinoembryonic antigen full length protein was purchased from Adcam (MA,USA). Mouse monoclonal capture CEA antibody (CEA mAb, clone no. M11147) and mouse monoclonal detection CEA antibody (CEA mAb, clone no. M11146) were purchased from Fitzgerald (MA, USA). Gold(III) chloride trihydrate ($HAuCl_4{\cdot}3H_2O$, 99.9+%), lyophilized 99% bovine serum albumin (BSA), terephthalaldehyde ($C_8H_6O_2$, 99%) and polyethylene oxide (PEG2000) were purchased from Sigma-Aldrich (WI, USA). Citric acid, trisodium salt dihydrate ($C_6H_5Na_3O_7{\cdot}2H_2O$, 99%) and aminopropyltriethoxy-silane (APTES, 99%) were purchased from Acros Organics (NJ, USA). Immunoreagents were dissolved in pH 7.4, 0.1 M phosphate buffered saline (PBS) (0.01 M phosphate, 0.14 M NaCl, 2.7 mM KCl).

2.2. Gold nanoparticle synthesis

Au NPs were synthesized via an adaptation of the well-established Frens and Turkevich method (Frens, 1973; Kimling et al., 2006). In this work, an inverse-order method was used. By exchanging the order of reagent addition, it is possible to increase the oxidation rate of sodium citrate and hence control the size and morphology, with a narrower size distribution than the standard Turkevich approach (Ojea-Jiménez et al., 2011). Narrow size distribution of the Au NPs is important for reproducibility and repeatability of the sensor responses. If particle distribution were not controlled, nanoparticle samples taken for different sensor determinations could have slightly different particle size distributions, and they could distribute differently on the surface of the SAW device as well. While the mass loading effect of the SAW device response is targeted for this application, viscoelastic properties of the adhered material also affect device response. A narrow distribution of the Au NPs will minimize these effects on the sensor response and contribute towards reproducible and repeatable sensor responses. The molar ratio of sodium citrate/$HAuCl_4$ is 6.8. Initially 25 mL of ultrapure (Milli-Q) water was heated up to 90 °C in an oil bath and then, 2.5 mL of 10.35 mg/mL sodium citrate was added. After the temperature of the solution was constant again, 1 mL of 5 mg/mL aqueous solution $HAuCl_4{\cdot}3H_2O$ was added and continuously heated for 15 min before cooling down to room temperature using an ice bath.

The optical absorption spectra of the gold colloidal solutions were measured using a Perkin Elmer Lambda 35 UV/Vis spectrophotometer (Perkin Elmer, USA). Dynamic light scattering (DLS) was performed on Zetasizer Nano ZS90 (Malvern, UK) to measure the average diameter of gold particles. The morphology of the synthesized gold particles was characterized by scanning electron microscopy (SEM, Hitachi S-800, Japan). An Agilent 8753ES network analyzer (Agilent, USA) was used to measure the SAW device responses. A syringe pump (Harvard Apparatus PHD 22/2000, USA) was used for the sample injection.

2.3. Preparation of the nanoprobes

The optimal dose of antibody for coating the gold particles were established first. Insufficient ratio of antibodies to colloidal gold particles could lead to instability, with consequent aggregation of the gold particles, which can be seen from the color change. The colloidal gold solution was first adjusted to pH 9 by adding 100 mM Na_2CO_3, and separated into 4 cells of 200 μL each. 1 mg/mL detection anti-CEA antibody solutions was added to each cell with varying volumes. After 5 min, 10 μL of 10% NaCl solution was added to each cell, and 1 min later, the color change of each cell was observed. The solution color in cell with 1 μL and 2 μL antibody turned from red to violet and the ones with 5 μL and 10 μL antibody solutions remained red. Thus, 5 μL/200 μL was determined to be the optimal ratio of antibody solution to colloidal gold solution. The results for different assembly ratios are shown in the Supplementary material.

After the optimal ratio was determined, the Au NPs were modified with detection anti-CEA antibody to prepare the nanoprobe conjugates as follows: 1 mL of colloidal gold solution was centrifuged and the supernatant was removed to concentrate the solution to 200 μL. After mixing, 100 mM Na_2CO_3 was added to the solution to adjust the pH of the solution to 9. Then, 25 μL of 1 mg/mL detection anti-CEA antibody was added and stored overnight at 4 °C after shaking for 20 min. In the next step, 5% PEG2000 was mixed with the assembled solution to reach a concentration of 0.5%, and stored for 1 h at 4 °C. To remove the excess antibodies, the gold nanoprobe solution was centrifuged, supernatant-removed and re-suspended with 500 μL of 0.1 mM phosphate buffered saline (PBS, pH 7.4) with 1% BSA, which was repeated 3 times. Finally, the nanoprobe solution were stored at 4 °C prior to further use.

2.4. Love wave sensor design and fabrication

Owing to the outstanding temperature performance and processing ease for ST-quartz compared to other piezoelectric crystals such as $LiTaO_3$ and $LiNbO_3$ (Zhou et al., 2013), and also the potential for