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Gold nanoparticle modified capacitive sensor platform for multiple marker detection

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ABSTRACT

The detection and quantification of cancer biomarkers in human blood is crucial to diagnose patients in the early stage of a disease. The recent advances in biosensor technology can improve detection by reducing the application time and cost without an invasive approach. In this study, a highly sensitive, novel nanoparticle-modified capacitive sensor was developed for the detection of cancer markers. The current work mainly focused on developing a surface modification protocol for achieving higher sensitivity using Au-NPs. An interdigitated electrode (IDE) transducer was modified using gold nanoparticles (Au-NPs) for signal enhancement, the platform was initially optimized with a small size IL-6 protein and the methodology was then applied for multiple marker detection with the aim of precise disease diagnostics. Carcinoembryonic antigen (CEA) and epidermal growth factor receptor (hEGFR) could be successfully detected in the concentration range of 20–1000 pg mL^{-1} while cancer antigen 15-3 (CA15-3) was detected in the range of 10–200 U mL^{-1}. These results show an increase of sensitivity by five-fold with respect to those not modified, demonstrating a highly sensitive and specific capacitive immunoassay that has a great potential for the use of early diagnosis of cancer disease.

1. Introduction

Capacitive sensors can be divided into two groups as faradaic and non-faradaic sensors depending on the transient current flow. In a faradaic process charges are transferred across an interface whereas transient currents can flow without addition of a redox charge transfer in non-faradaic processes. Therefore, redox species are alternately oxidized and reduced by the transfer of an electron to and from the metal electrode in faradaic capacitive sensors. Due to this, these kind of capacitive sensors require the addition of a redox-active species and DC bias conditions. On the other hand, additional reagent is not required in non-faradaic sensor and this behavior makes them more amenable to point-of-care applications [1]. The principle of the measurement depended on the changes in dielectric properties and charge distribution while antibody-antigen or probe-DNA/RNA complexes occurred on the electrode surface in non-faradaic case. In the event of a conformational change of a surface protein through binding of an analyte, this can be detected by capacitance measurements. The capacitance measurement can be realized through the measurement of the change in the capacitance between two metal conductors in near proximity to one another with the recognition element immobilized on IDEs. The detection principle of the sensor system is based on capacitive coupling of the excitation signal (conductivity and permittivity of medium) produced by IDE electrodes [2]. Thus, the electric field lines always penetrate into the accumulating medium (antigen-antibody complex) regardless of the position of the electrodes (parallel or co-planar). Depending on the geometric configuration of the electrodes, the electric field lines can penetrate deeper with wider electrode configuration [2]. Therefore, the capacitance of the IDE always depends on the geometry of the electrodes that is constant and the dielectric property of the medium. For interdigitated electrodes, the capacitance is defined with the following equation:

$$C = \varepsilon\varepsilon_o \frac{A}{d} \quad (1)$$

where, ε is the dielectric constant of the medium between the plates, ε_o (8.85419 pF/m) is the constant of permittivity of free space, A is the area of the plates and d is the distance between the plates. Immunoassays on the IDE transducer surface generally occur by the deposition of different biochemical layers (SAM, antibodies, Au-NPs and antigens) that increase the probe layer thickness, and thus, all biological samples have an arrangement of electric charge carriers [3]. These charges are displaced by an external electric field and polarized to neutralize the effect of the external electric field. This dielectric response of each type of protein over the frequency spectrum is unique in its characteristic [4]. Therefore, if a change occurs in the dielectric properties in the supplies between the plates, it leads to a change in the capacitance [5].

Interdigitated finger electrodes (IDE) have been used to obtain a larger sensor surface and with some modifications on IDE's they

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provide the direct detection of many entities including acetylcoline, toxin, oxygen bubbles, HIV and human IgG antibodies. A complex protein includes hydrophobic and hydrophilic regions, and the protein folds in a soluble media depending on this behavior. While a protein is immobilized on a solid surface and allowed to bind its analyte, a protein–analyte complex is formed. The change in conformation brought on by this interaction leads to an increase in molecular size of a protein–analyte complex. Hence, a local disturbance of the distribution of bound charges will occur at the dielectric interface, and these charges move under strong confinement and their local moment is termed as dipole moment [6]. Thus, increase in size of a protein–analyte complex leads to a relatively large permanent dipole moment which stimulate dielectric polarization on the IDE surface [5]. Therefore, the measured impedance/capacitance of the IDE varies with the relative change of the dielectric properties of the modified surface medium.

In most label-free faradaic-/optical-based biosensor systems, the nanoparticles were utilized to amplify the signal. Here, an electrochemical-based non-faradaic capacitive sensor was employed and developed for multiple cancer marker detection. In order to achieve higher sensitivity for determining very small sized analytes, a new solid-phase surface-modification protocol have been developed. In fact, we demonstrated recently that micro-sized magnetic beads can also be used as a solid-phase support material for determining very small sized analytes [7]. The surface coverage with the large sized magnetic beads and an extra process step of sandwich assay in the developed protocol hinder its further applications. Here, the sensor was modified with gold nanoparticles (Au-NPs) that have unique properties [8–11]. The Au-NPs modified sensor (IDE) surface provides stability for the immobilization of biomolecules that retain their biological activities (probably due to enhanced orientational freedom) which is extremely useful when preparing label-free impedimetric biosensors. Various characteristics of gold nanoparticles, such as their high surface-to-volume ratio, their high surface energy, their ability to decrease the distance between proteins and metal particles, and their ability to act as an electron-conducting pathway between prosthetic groups and the electrode surface, may facilitate electron transfer between redox proteins and the electrode surface [8]. In addition, it is noted that using covalent approach towards the directed self-assembly of gold nanoparticles from solution results in dense monolayer coverage of the particles on the IDE surface. The interaction between the gold nanoparticles and the IDE is mediated by a weak covalent bond. This allows the immobilization of dense networks of gold nanoparticles on IDE surface, which is of interest for use in label-free electrical detection system to achieve higher sensitivity by increasing the density of biological species within the constant sensor area.

Biosensors can be constructed by immobilizing the biomolecules by adsorbing them onto the nanoparticles, by cross-linking them with bi-functional agents such as glutaraldehyde, or by mixing them with the other components of composite electrodes [12]. Moreover, the nanoparticle surface can generate highly-active and large surface area. This enables binding of ultra low target concentrations and increases the density of biological species within the constant sensor area. In this work, the interdigitated capacitive transducer was modified with Au-NPs after SAM formation for signal amplification to detect trace amount of biomarkers in multiple cancer marker detection which has crucial role for precise cancer definition [13–16].

2. Materials and methods

2.1. Materials and reagents

Phosphate buffered saline (PBS) and 2× 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer were purchased from PAN BIOTECH GmbH, Germany and Fluka, Germany, respectively. Ethanolamine (99%), bovine serum albumin (BSA), human serum, mouse monoclonal antibody to human IL-6, human IL-6 antigen, thiourea, N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (N-hydroxy-2,5-pyrrolidinedione, NHS), sheep monoclonal antibody to human epidermal growth factor receptor (anti-hEGFR), human epidermal growth factor receptor (hEGFR) and human serum were purchased from Sigma-Aldrich (USA). CEA and CA15-3 antigens and their monoclonal antibodies were bought from Fitzgerald (USA). Carboxy encapsulated gold nanoparticles were purchased from NanoComposix (San Diego, CA). Doubly distilled water (dH_2O) was used throughout the experiments.

2.2. Preparation of Au-NP modified sensor platform

IDEs were patterned on silica (SiO_2) surface using image reversal technique. In this process, the metal layers were patterned using the dual tone photoresist AZ5214E. A 2-μm thick AZ5214E photo resist was used to create an inverse pattern of the mask design. Following this step, a very thin titanium (Ti) layer of ~20 nm size was layered to improve the adhesion of gold (Au) on the SiO_2 film by direct current (DC) sputter deposition, and about ~180 nm thick gold layer was deposited. The lift-off process was performed by washing away the sacrificial photoresist (AZ5214 E) in pure acetone. As a result, IDE array containing 24 gold interdigitated fingers were patterned. The dimension of each finger electrode was 800 μm in length, 40 μm in width.

The fabricated sensor chip was washed several times with ethanol and rinsed with sterile dH_2O. The cleaned surface was dried by nitrogen gun. The blank measurements were taken by Network Analyzer prior to any surface/bio-chemical treatment/application on the surface. The sensor surface was then coated with self-assembled monolayer (SAM) by immersing the sensor in 10 mM solution of thiourea for overnight incubation followed by rinsing with ethanol and dH_2O and then dried using nitrogen gun. The formation of SAM layer on the surface was confirmed by Fourier transform infrared spectroscopy (FT-IR). Carboxy encapsulated Au-NPs that have 5-nm size was prepared using the buffer solution in 27 μg mL^{-1} concentration [17]. After 8 h of incubation with Au-NPs onto the IDE, the modified surface was activated using 50 mM EDC and NHS and incubated for 3 h. The sensor platform was then washed with PBS and dH_2O. The impedimetric response of Au-NP modified IDE was measured using a Network Analyzer.

2.3. Antibody immobilization

Prior to the multiple marker detection assays for cancer biomarkers, the Au-NP modified sensor platform was optimized using IL-6-anti-IL-6 antigen-antibody pair as model analyte. For this, the Au-NP modified IDE surfaces of capacitors were immobilized by incubating 2.5 μL of 25 μg mL^{-1} IL-6 antibody in PBS buffer for 1 h. The sensor wafer was then washed with PBS and dried prior to the blocking step with ethanolamine. The non-reacted groups on the sensor surface were blocked by adding 5 μL of 100 mM ethanolamine on each IDE and incubated for 1 h. The sensor was then rinsed with PBS and sterile dH_2O, and dried with nitrogen gun prior to the measurements for antibody immobilization using a Network Analyzer. The analyzer was calibrated and triplicate measurements were then taken for each electrode for error analysis. The protocol developed for enhanced sensitivity was shown as schematic in Fig. 1a.

2.4. Capacitance measurements

To measure dielectric parameters (impedance/capacitance), a Karl-Suss (PM-5) RF Probe Station and an Agilent-8720ES S-parameter