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Label-free detection of cardiac biomarker using aptamer based capacitive biosensor

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Abstract

An aptamer based capacitive label-free biosensor was developed for the detection of a cardiac biomarker (C-reactive protein, CRP), based on charge distribution under the applied frequency by Non-Faradaic Impedance Spectroscopy. This capacitive biosensor is based on reagentless processing and developed using gold-interdigitated (GID) capacitor arrays functionalized with synthetic RNA-aptamers. The change in relative capacitance occurred by formation of RNA-CRP complex on GID-capacitors against the applied AC electrical frequency (50-350 MHz) was measured. The binding affinity (K_d) of the RNA-aptamer with CRP was determined and the results showed that at frequencies of 208 and 306 MHz the strong binding occurred with K_d = 1.6 μM and 3.4 μM, respectively. The dynamic range for CRP was determined to be within 100-500 pg/ml. Our results demonstrate the sensitive detection of RNA-protein complex on GID-capacitors under the applied electric field, which can be extended to other disease protein biomarkers for the development of electrical biosensors.

Keywords: Label-free; capacitive biosensor; aptamer; cardiac marker

1. Main text

The aptamers are short, single stranded DNA or RNA oligonucleotides that can bind to their targets and offer specific properties, which favor them as new biorecognition elements for biosensors [1]. Until now, the different nature of these nucleic-acid recognition elements and their protein targets, and the unique properties of aptamers, indicate great promise for designing innovative sensing protocols [1]. Most aptamer-based biosensors reported to date rely on standard sandwich bioaffinity assays in connection to common enzyme, fluorophore, or nanoparticle tracers. Recently, electrochemical aptamer-based biosensors (faradaiac type) has been reported on different platform to detect specific proteins as disease biomarkers [2]. In some cases, long incubation time is required due to the slow diffusion of analyte through an unstirred layer to form the immunocomplex. These disadvantages limited greatly their application in disease diagnosis. Here, we describe a novel aptamer based capacitive label-free biosensor for monitoring transducing aptamer–CRP recognition events based on charge distribution under the applied frequency

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by Non-Faradaic Impedance Spectroscopy (NFIS). The measuring principle of these sensors is based on simple changes in dielectric properties, charge distribution, and conductivity change when a ligand-target complex formed on the surface of an electrode. Capacitive affinity biosensors can be constructed by immobilizing recognition elements, such as antibodies or aptamers in thin layers on the electrodes, and measuring changes in the dielectric/surface properties when an analyte binds. For providing larger sensor surface, conductors can be made into a pattern of interdigitated fingers.

The capacitance between the interdigitated electrodes can then be described by the basic capacitance equation

$$C = 2n\epsilon\epsilon_0 A/d \quad (1)$$

where ϵ is the dielectric constant of the medium between the plates, ϵ_0 is the permittivity of free space, A is the area of the electrodes and d is the distance between the two electrodes, n being the number of electrodes.

In this study, CRP is chosen as a target as it is one of the plasma proteins known as acute-phase proteins under CVR conditions. CRP can rise as high as 1000-fold because of inflammation induced by infection or injury that often lead to CVR [3]. Recent research suggests that patients with elevated basal levels of CRP are at an increased risk of diabetes hypertension as well as cardiovascular disease [3].

GID array electrodes were patterned on SiO_2 surface using image reversal technique. The dimension of each electrode was 800 µm in length, 40 µm in width with a distance between two electrodes of 40 µm. Each capacitor sensor contained 24-interdigitated electrodes with a total area of 3×3 mm. A series of GID-capacitor arrays immobilized with thiol-RNA aptamer (10 µM) were used for the binding assays against different concentrations of CRP target (0-600 pg/ml). Schematic diagram of arrays of GID-capacitor chips functionalized with thiolated RNA-aptamer and binding of the target CRP is shown in Fig. 1.

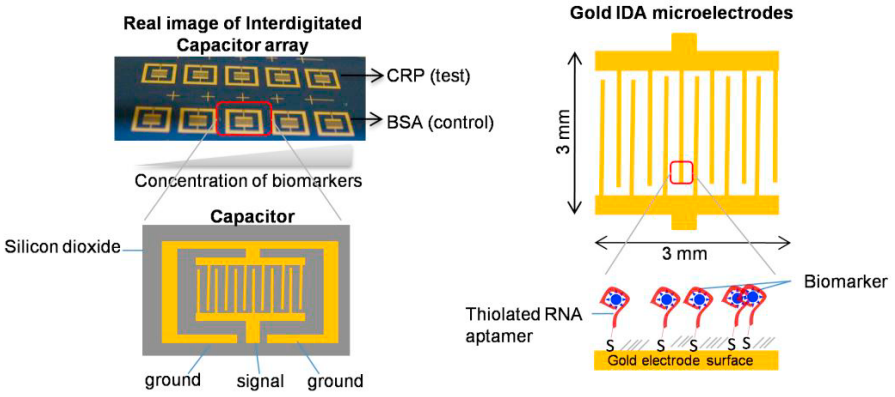

Fig. 1. Schematic diagram of arrays of GID-capacitor chips functionalized with thiolated RNA-aptamer and binding of the target CRP.

The relative capacitance variations were calculated from the data obtained within 50-400 MHz frequency range under standard assay conditions. To calculate the dissociation constants, the values were fitted by the non-linear regression analysis, assuming a Langmuir adsorption isotherm, the change in relative capacitance was then directly related to the RNA aptamer binding to CRP. The calculated K_d values at 208 and 306 MHz frequencies were 1.6 and 3.4 µM, respectively and shown in Fig. 2. These results indicated that strong binding of the RNA aptamer on the capacitor surface occurred at 208 MHz compared to those found at 306 MHz frequencies.

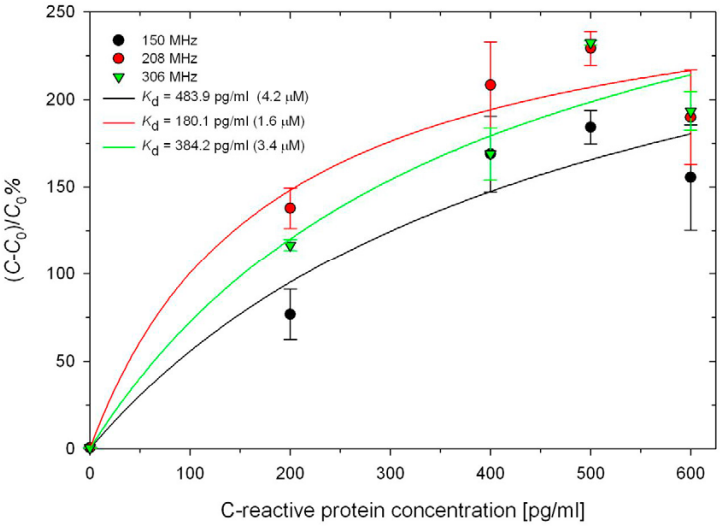

Fig. 2. Concentration dependent change in relative capacitance occurred after RNA aptamer–CRP complex formation on GID-capacitors against varying CRP concentrations at three different frequencies as shown in legend.

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