A novel interdigitated capacitor based biosensor for detection of cardiovascular risk marker

Anjum Quershi [a], Yasar Gurbuz [a,*], Weng P. Kang [b], Jimmy L. Davidson [b]

[a] Sabanci University, Faculty of Engineering and Natural Sciences, Tuzla, 34956 Istanbul, Turkey
[b] Department of Electrical and Computer Engineering, Vanderbilt University, Nashville, TN 37235, USA

ARTICLE INFO

Article history:
Received 24 August 2009
Accepted 28 August 2009
Available online 2 September 2009

Keywords:
Nanocrystalline diamond film
Antibody
Capacitive
C-reactive protein
Biosensor

ABSTRACT

C-reactive protein (CRP) is a potential biomarker whose elevated levels in humans determine cardiovascular disease risk and inflammation. In this study, we have developed a novel capacitive biosensor for detection of CRP-antigen using capacitor with interdigitated gold (GID) electrodes on nanocrystalline diamond (NCD) surface. The NCD surface served as a dielectric layer between the gold electrodes. GID-surface was functionalized by antibodies and the immobilization was confirmed by Fourier transform spectroscopy (FT-IR) and contact angle measurements. The CRP-antigen detection was performed by capacitive/dielectric-constant measurements. The relaxation time and polarizability constants were estimated using Cole–Cole model. Our results showed that the relaxation time constant (τ) of only CRP-antibody was within 10^{-16}–10^{-13} s, which was increased to 10^{-11} s after the incubation with CRP-antigen, suggesting that the CRP-antigen was captured by the antibodies on GID-surface. In addition, polarizability constant (m) of CRP was also increased upon incubation with increasing concentration of CRP-antigen. Our results showed that the response of GID-NCD-based capacitive biosensor for CRP-antigen was dependent on both concentration (25–800 ng/ml) as well as frequency (50–350 MHz). Furthermore, using optimized conditions, the GID-NCD based capacitive biosensor developed in this study can potentially be used for detection of elevated levels of protein risk markers in suspected subjects for early diagnosis of disease.

1. Introduction

C-reactive protein (CRP) is one of the plasma proteins known as acute-phase proteins and its level rise dramatically during inflammatory processes occurring in the body. This increment is due to a rise in the plasma concentration of IL-6, which is produced predominantly by macrophages as well as adipocytes (Pepys and Hirschfield, 2003; Lau et al., 2005). CRP can rise as high as 1000-folds with inflammation. Conditions that commonly lead to marked changes in CRP include infection, trauma, surgery, inflammatory conditions, advanced cancer and moderate changes occur after strenuous exercise, heatstroke, and childbirth (Pepys and Hirschfield, 2003; Casas et al., 2008). CRP was found to be the only marker of inflammation that independently predicts the risk of a heart attack (Pepys and Hirschfield, 2003; Hansson, 2005; Casas et al., 2008). The risks for cardiovascular diseases defined by the American Heart Association (AHA) and the Center for Disease Control and Prevention (CDC) are low for a CRP concentration below 1.0 mg/l, moderate for a CRP level from 1.0 to 3.0 mg/l, and high for concentrations over 3.0 mg/l (Yang et al., 2009).

In order to measure a lower CRP concentration in clinical laboratories, an ELISA protocol is commonly used with a detection limit as low as 1.0 mg/l (Ledue and Rifai, 2001). An immunosensor was designed using potential and ac impedance methods to obtained a linear detection range of 5–25 mg/l CRP (Zhu et al., 2003). Recently, there are several advancements for measuring CRP has been reported. For example, a CRP assay by using a magnetic permeability detector has been reported with a detection limit of 0.2 mg/l (Kriz et al., 2005). A label-free assay using surface plasmon resonance (SPR)-based technology was demonstrated with a detection limit of 2–5 mg/l (Meyer et al., 2006). Furthermore, a novel magnetic biosensor for CRP antibodies was developed and showed a linear detection range from 25 μg/l to 2.5 mg/l (Meyer et al., 2007). In an another approach, magnetic nanoparticles conjugated with labeled anti-CRP-antibody were used to perform an immunoassay that is shown to have a detection limit of 0.12 mg/l (Tsai et al., 2007). The detection limit was improved to 0.005 mg/l using an RNA-based aptasensor by optical based SPR method (Bini et al., 2008). An integrated microfluidic system was developed using the microfluidic chip incorporated with magnetic beads coated with specific DNA aptamers and the detection limit was improved from 0.125 to 0.0125 mg/l (Yang et al., 2009). However, in order to obtain this lower limit of detection, the acridinium-labeled antibodies were used for sandwich type chemiluminescence measurements.

* Corresponding author. Tel.: +90 216 483 9533; fax: +90 216 483 9550.
E-mail address: yasar@sabanciuniv.edu (Y. Gurbuz).

Nevertheless, all these methods that still require labor-intensive processing and large and expensive equipments for measurement of the CRP that requires expertise.

Capacitance measurement could be a useful tool for immunoassays (Christine et al., 2001). The attraction of affinity-based capacitive sensors is that they are able to determine the analyte directly in a sample with no or very little sample preparation. The measuring principle of these sensors is based on changes in dielectric properties, charge distribution, and conductivity change when an antibody–antigen complex formed on the surface of an electrode. Capacitive affinity biosensors can be constructed by immobilizing recognition elements, such as antibodies 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 $C = 2n\varepsilon\varepsilon_0 A/d$, 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 (12 in this study) and finally the factor 2 in this equation represent to each electrode forming two capacitors. Thus, when there is a change in the dielectric properties of the material between the plates, a change in the capacitance will occur and it is correlated to the bound antigen molecules captured by antibodies on the surface, as well as between the electrodes.

In a complex protein, positive and negative charges are constituted from the ionizable side chains of acidic and basic amino acid present in the protein structure. The simplest molecular dipole consists of a pair of opposite electrical charges with magnitude of $+q$ and $-q$ and separated by r, vector distance. The molecular dipole moment m is given by the equation $m = qr$. Each type of polar or polarizable entity will exhibit its own characteristic response to the imposed electric field. When 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. This increase in size of a protein–analyte complex therefore leads to a relatively large permanent dipole moment. The relaxation time and polarizability constants can thus be estimated (Cole and Cole, 1941).

In this paper, to our knowledge, for the first time, a new capacitive immunosensor was developed, based on gold interdigitated electrodes fabricated on GID-NCD surface to detect CRP. Using CRP-antibody as the model ligand/substrate, a direct detection of CRP by capacitance/dielectric measurements was demonstrated using a heterostructure of GID-NCD surface covalently bound with CRP antibodies on GID region. When the immobilized CRP-antibody interacts with CRP-antigen, the interaction leads to the change in thickness of the dielectric layer on GID-NCD surface, and induces change in capacitance which can directly be related to detect the antigen.

2. Materials and methods

2.1. Reagents and materials

Monoclonal antibodies and purified antigen, C-reactive protein was purchased from Fitzgerald Industries International (Concord, MA, USA). 3-Mercaptopropionic acid, *N*-(3 dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC), and *N*-hydroxysuccinimide (*N*-hydroxy-2,5-pyrrolidinedione, NHS) were obtained from Sigma–Aldrich (Steinheim, Germany). PBS and Tween 20 were purchased from Sigma (USA). All other reagents and solvents were of analytical grade and the doubly distilled water was used throughout the experiments.

2.2. Synthesis of NCD film and characterization

NCD films were formed with the process of the $CH_4/H_2/N_2$ gases in a Microwave Plasma Enhanced Chemical Vapor Deposition (PECVD), employing growth rate reduction conditions and using an ASTeX® reactor equipped with a 2.45 GHz microwave generator. The pre-treatment process of the silicon (1 0 0) substrate consisted of mechanical polishing of the surface using diamond powder (2.5 μm size) followed by ultra-sonication with diamond powder of 5–20 nm size in acetone solution to augment diamond nucleation. A gaseous mixture of $CH_4/H_2/N_2$ with flow rates of 15/8/190 sccm, respectively, was introduced into the CVD system at a pressure of 20 Torr with the substrate temperature being 800 °C, and microwave power at 550 W. The growth rate of the diamond deposition process was controlled to be 0.1 μm/h, in order to achieve nm-scaled grains of diamond. Scanning electron (JSM-5600LV-SEM) and optical micrographs of the nanocrystalline diamond surfaces were carried for characterization. These NCD films were used for GID array patterning for fabrication of capacitors.

2.3. Fabrication of GID array electrodes on NCD surface for fabrication of capacitors

The GID array capacitors (GID-NCD chips) were fabricated by following steps. First, the GID array electrodes were patterned on NCD film surface using image reversal technique. In this process the metal layers were patterned using the dual tone photoresist AZ 5214 E. Very thin tungsten layer of 50 nm size was DC sputter deposited on the NCD film, which was used to improve the adhesion of gold on substrate. Next, gold particles of 500 nm size were deposited using DC sputtering. Following this step, the gold layer was patterned by image reversal technique with the help of a mask. Length of each electrode was 750 μm and a width of 25 μm and a distance between two electrodes was 25 μm. A 40 μm deep SU8 wells were patterned over the GID structure for easing the antibody immobilization on the sensor structure. As a result, each GID array on a capacitor contained 24 GID fingers. A series of such capacitors were fabricated for detection of CRP-antigens. The surface characterization was performed using a nanoscope AFM (Nanoscope) with the tapping mode and by optical micrographs.

2.4. Immobilization of CRP antibodies on GID electrodes

The immobilization of CRP-antibodies on GID electrode surface of capacitors was carried out in a two step reaction. First, the chips were immersed in a 10 mM solution of mercaptopropionic acid (MPA) at room temperature for 24 h and a self-assembled monolayer (SAM) was formed (Riepl et al., 1999). The SAM layer of MPA on GID electrodes was thoroughly rinsed with distilled water and dried over pure nitrogen gas. The formation of SAM is a spontaneous reaction by adsorption of MPA on gold surface by sulfide linkage as shown in the following chemical reaction (Warakorn et al., 2006).

$$RSH + Au \rightarrow RS^{-}Au^{+}\cdot Au + 1/2H_2 \quad (1)$$

or disulfide

$$RS{-}SR + Au \rightarrow RS^{-}Au^{+}\cdot Au \quad (2)$$

The affinity between sulfur and gold atoms is shown to be extremely high, and the resulting SAMs are highly stable in air, water, and organic solvents at room temperature (Warakorn et al., 2006; Chaki and Vijayamohanan, 2002; Wadu-Mesthrige et al., 2000). In the second reaction, the CRP antibodies were immobilized

on SAMs through covalent binding. For this, the carboxylic groups of MPA (SAM) on GID electrodes were activated by adding 0.05 M of EDC and 0.03 M of NHS in water for 5 h. The activated GID-electrode was then incubated with 20 μl of 100 μg/ml CRP antibodies in PBS (as a model sample) for 1 h at room temperature. The GID-electrode surface immobilized with CRP antibodies was washed thrice with PBS buffer followed by double distilled water. The free/unoccupied carboxyl groups on GID electrodes was blocked by adding 100 mM ethanolamine in PBS buffer and incubated for 2 h at 4 °C and washed again with PBS buffer. Finally, the immobilization of antibodies was confirmed by IR spectrum using a THERMO (Nicolet) 6700 Model FT-IR spectrometer and contact angle measurements.

2.5. Detection of CRP-antigen and characterizations

A series of capacitors were used for detection of different concentrations of CRP-antigen (at least two capacitors for the detection of each concentration of CRP). Detection process was performed by incubating CRP-antigen on GID electrode region of a capacitor. Initially, a series of CRP-antigen concentrations (0–1000 ng/ml) were prepared in PBS buffer. The capacitors were then incubated for 1 h with different CRP-antigen concentrations in 2 μl volume each. The dielectric parameters (impedance/capacitance) were measured before and after the antigen treatment. First, the dielectric parameters were measured with (a) blank capacitor, (b) capacitor after SAM formation, (c) after surface activation, (d) after CRP-antibody immobilization, and compared the results with (e) after capturing of different concentrations (25, 100, 500, 800 and 1000 ng/ml) of CRP-antigen by antibodies on capacitors. The dielectric parameters (impedance/capacitance) were measured in the frequency range 50 MHz–1 GHz using Network Analyzer (Karl-Suss PM-5 RF Probe Station and Agilent-8720ES). Network analyzer was calibrated using SOLT (short-open-load-through) method. Dielectric constant and conductivity were calculated from measurements of the sample capacitance and resistance. A negative control experiment was also conducted under standard conditions using bovine serum albumin (BSA) as a non-specific protein in place of CRP-antigen.

Dielectric constant and conductivity values were extracted from the data measurements at frequency (f) of 50–425 MHz range for plotting. Further, the relaxation time and polarizability constants were estimated using Cole–Cole model (Cole and Cole, 1941).

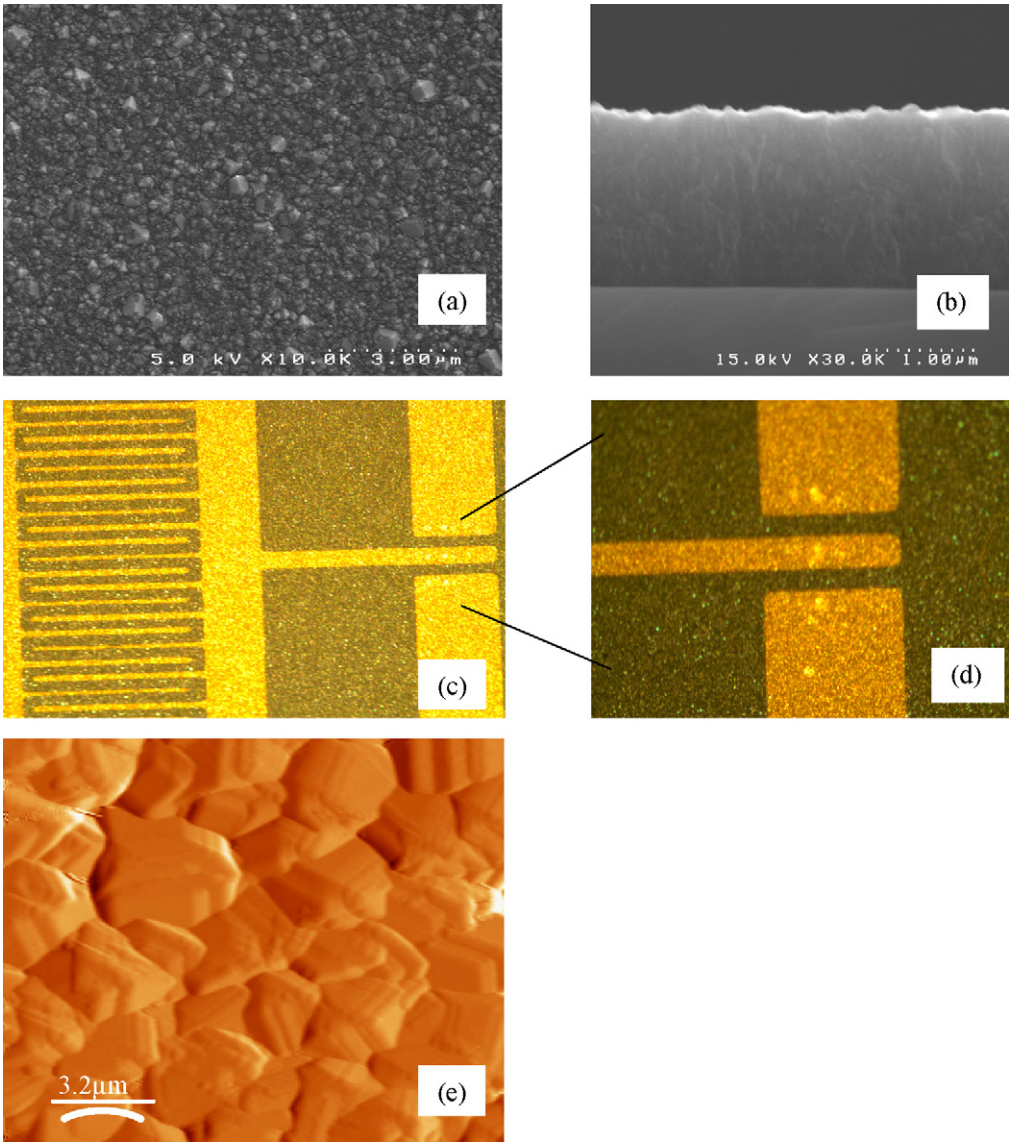

Fig. 1. Scanning electron micrographs of (a) the NCD film on silicon substrate and (b) cross-sectional image; optical micrographs of (c) GID-NCD surface and (d) a magnified portion of sensor surface (dimension of the electrode was 750 μm × 25 μm) with spacing between two electrodes was 25 μm; and (e) AFM image of the sensor surface.

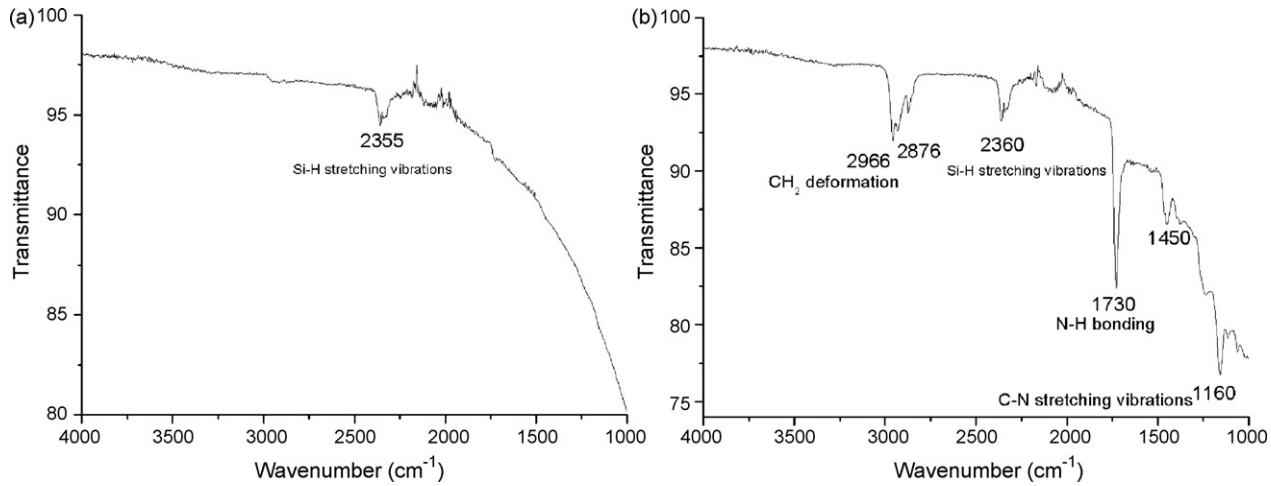

Fig. 2. FT-IR spectrum of the gold-interdigitated on nanocrystalline diamond surface (capacitor): (a) before (blank surface) and (b) after the antibody immobilization.

Average values of dielectric properties obtained from at least two capacitors for each antigen concentration was plotted and the standard deviations are shown as errors.

3. Results and discussion

In this study we report on the development of a novel capacitive-based biosensor for detection of CRP-antigen. Here, the antibodies specific for CRP-antigen were used as bio-recognition elements in this capacitive-based biosensor. For this, initially GID-array capacitors were fabricated on NCD films. The NCD surface allowed a good dielectric layer between the GID electrodes. The antibodies were immobilized on GID-surface of the capacitors. The CRP-antibody functionalized capacitors were incubated with a series of CRP-antigen concentrations. As a result, the signal generated due to the formation of antibody–antigen complex on capacitor surface was determined by measuring the dielectric properties.

3.1. Characterization of GID-NCD surface

The NCD films were prepared on silicon substrates by CVD technique onto which the GID arrays to be patterned. The surface characteristics was observed by SEM and the diamond surface was found to be homogenous, flat and nonporous (Fig. 1a). Further, the surface texture showed a thickness of ~1.5 μm NCD film on silicon substrate (Fig. 1b). The GID electrode arrays were then patterned as described in materials and methods. The distribution of gold nanoparticles on NCD film was found to be homogenously well distributed as shown in optical micrographs and an AFM image (Fig. 1c–e). The thickness of sputtered gold was about 500 nm on the NCD film surface.

3.2. Confirmation of antibody immobilization on GID arrays of capacitors

FT-IR spectrum of the GID-NCD surface was recorded before and after the antibody immobilization. The FT-IR spectra of GID-NCD before immobilization showed only an absorption peak at 2200–2400 cm^{-1} (2360 cm^{-1}) wave number that represents the stretch vibration of Si–H groups of NCD on silicon substrate (Fig. 2a). The FT-IR spectra after the antibody immobilization showed the presence of N–H bonding (1730 cm^{-1}) on GID arrays, indicating that antibodies were successfully immobilized (Fig. 2b). The less intense peak at 1450 cm^{-1} shows the presence of normal amine. The peak at 1160 cm^{-1} shows the C–N stretch.

Further, contact angle was measured on self-assembled monolayer formed on the GID-NCD surface before and after the antibody treatment. The hydrophilicity brought on by SAM formation followed by antibody immobilization was confirmed by its contact angle of 73° and 60°, respectively. This result also showed that the antibody immobilization on the surface increased the hydrophilicity of the surface.

3.3. Detection of CRP-antigens using CRP-antibody functionalized capacitors

A series of capacitors functionalized with CRP antibodies were incubated with different concentrations of CRP-antigen (0–1000 ng/ml). The capturing of CRP-antigen was measured by dielectric properties using Network analyzer (Karl-Suss PM-5 RF Probe Station and Agilent-8720ES). The variation in the values of the dielectric constants as a function of frequency was plotted (Fig. 3). Further analysis was performed by plotting the values of dielectric constants against different concentrations of CRP-antigen

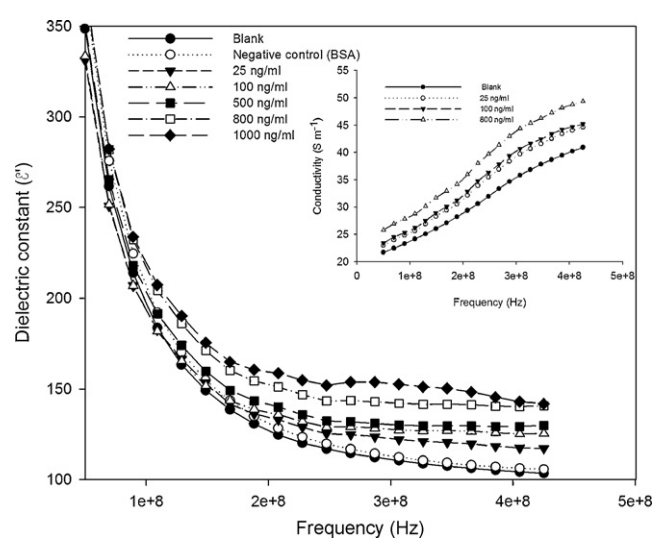

Fig. 3. The change in dielectric constant values of CRP-antigen–antibody complex on GID-NCD surface (capacitor) as a function of frequency (50–425 MHz). The CRP-antibodies immobilized GID-NCD-capacitors were incubated with different concentrations of CRP-antigen (0–1000 ng/ml) and dielectric properties were measured. A negative control was performed with BSA protein (800 ng/ml) that showed no response. The inset figure shows variation in the conductivity values of antigen–antibody complex on capacitor surface as a function of frequency.

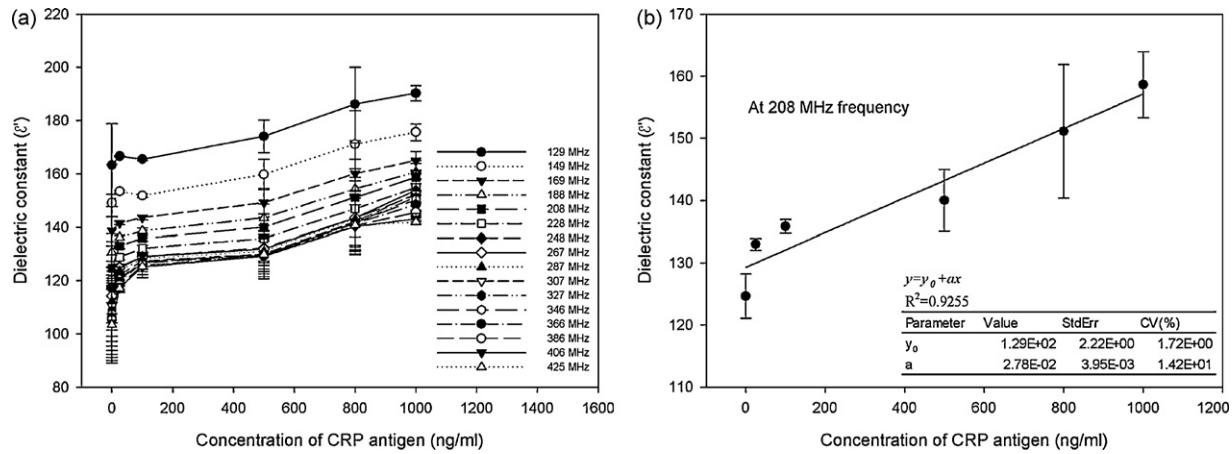

Fig. 4. The change in dielectric constant values of CRP-antibody–antigen complex on capacitor surface as a function of different concentrations of CRP-antigen at (a) different frequencies as shown in the figure legend and (b) at a specific frequency of 208 MHz.

(Fig. 4a and b). It was observed from the above figures that dielectric constant passed through dielectric dispersion and decreased with frequency (Grant, 1983). The conductivity was increased with the increasing CRP-antigen concentration at a constant frequency (see inset of Fig. 3). The data points of the dielectric change for linear fit at one of the frequencies was plotted against the concentrations of antigen and the coefficient of variation (CV) was found to be within 14%. Fig. 4b showed the concentration versus dielectric change at a frequency of 208 MHz was linear over 25–1000 ng/ml antigen concentration range (R^2 = 0.9255 with CV(%) of 1.72) (Fig. 4b).

Protein molecules, such as CRP-antigen and CRP-antibodies are composed of one or more polypeptide chains folded in a complex and fractural geometry. Polypeptide bonds, amino acid side chains and solvent molecules are organized at different levels to control secondary and three-dimensional structure of proteins through the formation of peptide bonds as shown in the reaction (see Supplementary information Fig. S1).

The N–C bond in the peptide units has a partial double bond character, so that the six atoms C_α NHCO C_α are coplanar. In addition, the C=O bond is itself polar, so that the peptide bond possesses a permanent dipole moment. Since each peptide unit possesses a permanent dipole moment, polypeptide chains take the form of strings of connected dipoles. The drop in values of dielectric constant with frequency was possibly because of the rotational relaxation of the protein molecules (β dispersion) (Pethig and Kell, 1987). In this study, the relaxation time for CRP-antibody alone was in the range 10^{-16}–10^{-13} s. This relaxation time was found to be elevated to 10^{-11} s when a series of CRP-antigen concentration was exposed. It was found that the larger molecule of a protein has a greater relaxation time (Pethig and Kell, 1987; Song, 2002; Dobrikova et al., 2007). Our results suggest that the CRP-antigen was captured by CRP-antibodies present on the surface of the heterogeneous sensor structure. As a result, the CRP-antigen–antibody complex becomes larger in molecular size than antibody alone, and therefore, the increase in relaxation time was correlated to the increase in the size of a protein molecule. The impedance equivalent network circuit based on Cole–Cole model (Cole and Cole, 1941) of GID-NCD sensor is given by the following equations:

$$Z(\omega) = Z(o)\left\{1 - m\left[1 - \frac{1}{1 + (j\omega\tau)^c}\right]\right\} \quad (3)$$

$$R_o = Z(o) \quad (4)$$

$$R_\infty = Z(o)[1 - m] \quad (5)$$

where R_∞ is the high frequency impedance, R_o is the low frequency impedance, m is the polarizability constant, and τ is the relaxation time constant. Further, the ploarizability constant (m) was calculated before and after the formation of CRP-antibody–antigen complex by fitting the values of impedance using the Eqs. (3)–(5). The polarizability constant value of only antibody on the sensor surface was calculated to be 0.5093, and this value was increased to 0.766 after incubation with CRP-antigen (Table 1).

The increase in the value of polarizability constant can be related to the rotational motion of the protein molecule upon binding of antibodies to CRP-antigen (Pethig and Kell, 1987; Schwan, 1968). It was also observed that the dielectric dispersion $\Delta\varepsilon' = (\varepsilon'_s - \varepsilon'_\infty)$ for control was lower than the antigen treated sample. The changes in the value of $\Delta\varepsilon'$ were attributed to change in shape and volume of protein molecules (Hasted, 1973). It is clear from our results that the values of conductivity increased, which accompanied by a decrease in the values of dielectric constant (see Fig. 3). Our results showed that the response of this capacitive-based sensor for CRP-antigen protein was dependent on concentration in a range 25–800 ng/ml of CRP-antigen as well as frequency at a range 50–350 MHz (Fig. 3). However, at a particular frequency (for e.g., 208 MHz), the linearity was extended from 800 to 1000 ng/ml (Fig. 4b), but at higher frequency (for e.g., 400 MHz), the maximum limit was only up to 800 ng/ml. The concentration and frequency above 800 ng/ml and 350 MHz, respectively showed no increase in response by this sensor system (Figs. 3 and 4a). This was possibly because of saturation of antibody binding sites on the sensor surface. In addition, the sensor surface was bio-functionalized with a

Table 1
Values of dielectric dispersion and polarizability constants for blank (no antigen but contains only antibodies on sensor surface) and tests with different concentration of CRP-antigen.

Parameter	CRP-antigen concentration (ng/ml)					
	0	25	100	500	800	1000
[a]Dielectric dispersion ($\Delta\varepsilon'$)	5951.43	5186.56	5000.31	5120.99	5448.04	5096.36
Polarizability constant (m)	0.5093	0.5305	0.5799	0.6016	0.7143	0.766

[a] The dielectric despertion values presented in the table are for 24 fringes on each capacitor.

constant amount of CRP-antibody (100 μg/ml). It is clear that there are limited binding sites on CRP-antibodies and thus the limitation of CRP-antigen binding capacity. Further, a negative control experiment was performed by using BSA protein (800 ng/ml) to ascertain that no non-specific signal occurred on a capacitor chip coated with CRP-antibodies. The results showed that BSA did not bind to antibodies on the chip surface (Fig. 3).

Several detection methods have been employed for sensitive detection of CRP-antigen. For example, the detection of CRP reported by electrochemical method shown to be in a range 5–25 mg/l (Zhu et al., 2003), magnetic permeability immunoassays with a lower detection limit of 0.2 mg/l (Kriz et al., 2005), by SPR in a range 2–5 mg/l (Meyer et al., 2006), and magnetic biosensor with a broad range of 25 μg/l–2.5 mg/l (Meyer et al., 2007). The most sensitive techniques of all the above was by ELISA with a lowest detection limit of 1 mg/l (Ledue and Rifai, 2001) and by using specific DNA aptamers through labeled CRP-antibodies and measuring chemiluminescence was 0.125–0.0012 mg/l (Yang et al., 2009). However, the methods reported in the literature are often expensive that are either sandwich type (more prone to cross-reactivity) or at least required a fluorophore for sensitive detection. Therefore, we here report for the first time, by use of a capacitor based biosensor, it was possible to detect CRP in a range 0.025–0.8 mg/l. The advantage of the proposed capacitor based detection of CRP over other methods is being its label-free nature, ease of use, less expensive, required minimum sample preparation, and direct signal readout in the form of electrical signal. All these cannot be offered by different approaches, such as SPR or sandwich type fluorescence detection. Above all, the optical sensors (SPR), chemiluminescence, or ELISA require lab based equipment or chemical fluorophores to read the signal (Bini et al., 2008; Yang et al., 2009). Further, under optimized assay conditions, the capacitive biosensor can be applied to detect other disease markers for testing with real serum samples. It should be noted that the dielectric layer (NCD) between the gold electrodes and their geometry contributes to improve the analytical performance. The use of NCD as a dielectric material prevents physical adsorption of constituents, masking and interference in dielectric signal. Furthermore, the optimization of the geometry of GID electrode and dielectric material for the fabrication of a capacitor chip would allow enhancing the sensitivity of capacitive biosensor.

4. Conclusion

In summary, we have developed a novel capacitive biosensor for detection of CRP-antigen, using interdigitated gold electrodes/nanocrystalline diamond capacitive structure, immobilized with human CRP-antibodies. The response and sensitivity of this capacitive-based biosensor for CRP-antigen was dependent on both concentration and applied frequency. The values of relaxation time (τ) and polarizability constants (m) of CRP were increased upon incubation with increasing concentration of antigen suggesting that the CRP-antigen was captured by the antibodies on the sensor platform. The dynamic detection range using optimized conditions for a given antibody concentration (100 μg/ml) was found to be in the range 25–800 ng/ml of CRP-antigen. This range falls within the concentration levels of CRP-antigen in a cardiovascular disease risk conditions. The sensitivity can be greatly improved by manipulating the surface area of capacitive sensor as well as the antibody concentration for immobilization. However, there are limitations with respect to the application of capacitive-based biosensors as a tool to specifically identify macromolecules in real serum samples. In physiological solutions, ions, such as sodium and chloride, create large conductive losses. Furthermore, the ions create a sheath around the polar groups of the macromolecules that may mask the desired signal and the macromolecule tends to blur the dielectric relaxation spectrum over a large frequency.

Nevertheless, the capacitive biosensor developed in this study is an inexpensive tool because it does not require expensive fluorescent dyes, reagents, and sophisticated instruments. It is a versatile technique that can be potentially applied for detection of elevated CRP levels in suspected subjects for early diagnosis of cardiovascular disease. Finally using optimized conditions, the capacitive biosensor can also be applied for detection of various other diseases using novel biomarkers or probes for diagnosis or detection of environmental contaminants.

Acknowledgments

We thank Bulent Koroglu and Mick Howell for their valuable contribution to the processing of devices. We also thank the Scientific and Technological Research Council of Turkey (TUBITAK) for the financial support for this project under the contract number 107E014 and title "RF Transmitter–Based Transducer for Biosensor Applications."

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2009.08.043.

References

Bini, S., Centi, S., Tombelli, M., Minunni, M., Mascini, 2008. Anal. Bioanal. Chem. 390, 1077–1086.
Casas, J.P., Shah, T., Hingorani, A.D., Danesh, J., Pepys, M.B., 2008. J. Intern. Med. 264, 295–314.
Chaki, N.K., Vijayamohanan, K., 2002. Biosens. Bioelectron. 17, 1–12.
Christine, B., Bjarni, B., Gillis, J., 2001. Electroanalysis 13 (3), 173–180.
Cole, K.S., Cole, R.H., 1941. J. Chem. Phys. 1, 341–351.
Dobrikova, A.G., Dimitrov, M.I., Taneva, S.G., Petkanchin, I.B., 2007. Colloids Surf. B: Biointerfaces 56, 114–120.
Grant, E.H., 1983. Dielectric Properties of Biological Tissue and Cells in RF, an MW Frequencies. Plenum Press, New York.
Hansson, G.K., 2005. N. Engl. J. Med. 352, 1685–1695.
Hasted, J.B., 1973. Aqeous Dielectrics. Chapman and Hall Ltd, London.
Kriz, K., Ibraimi, F., Lu, M., Hansson, L.O., Kriz, D., 2005. Anal. Chem. 77, 5920–5924.
Lau, D.C., Dhillon, B., Yan, H., Szmitko, P.E., Verma, S., 2005. Am. J. Physiol. Heart Circ. Physiol. 288, 2031–2041.
Ledue, T.B., Rifai, N., 2001. Clin. Chem. Lab. Med. 39, 1171–1176.
Meyer, M.H.F., Hartmann, M., Keusgen, M., 2006. Biosens. Bioelectron. 21, 1987–1990.
Meyer, M.H., Hartmann, M., Krause, H.J., Blankenstein, G., Mueller-Chorus, B., Oster, J., Miethe, P., Keusgen, M., 2007. Biosens. Bioelectron. 22, 973–979.
Pepys, M.B., Hirschfield, G.M., 2003. J. Clin. Invest. 111, 1805–1812.
Pethig, R., Kell, D.B., 1987. Phys. Med. Biol. 32, 933–970.
Riepl, M., Mirsky, M., Novotny, I., Tvarozek, V., Rehacek, V., Wolfbeis, O.S., 1999. Anal. Chim. Acta 392, 77–84.
Schwan, H.P., 1968. Ann. N. Y. Acad. Sci. 148, 191–209.
Song, X., 2002. J. Chem. Phys. 16, 9359–9363.
Tsai, H.Y., Hsu, C.F., Chiu, I.W., Fuh, C.B., 2007. Anal. Chem. 79, 8416–8419.
Wadu-Mesthrige, K., Amro, N.A., Liu, G.Y., 2000. Scanning 22, 380–388.
Warakorn, L., Kanatharana, P., Mattiasson, B., Asawatreratanakul, P., Thavarungkul, P., 2006. Biosens. Bioelectron. 22, 233–240.
Yang, Y.N., Lin, H.I., Wang, J.H., Shiesh, S.C., Lee, G.B., 2009. Biosens. Bioelectron. 24, 3091–3096.
Zhu, J.-J., Xu, J.-Z., He, J.-T., Wang, Y.-J., Miao, Q., Chen, H.-Y., 2003. Anal. Lett. 36, 1547–1556.