Proceedings of the Eurosensors XXIII conference

A new nanocrystalline diamond-based biosensor for the detection of cardiovascular risk markers

Anjum Quershi[1], Kallempudi, S. Saravan[1],Yasar Gurbuz[1]*, Mick Howell[2], Weng P. Kang[2], and Jimmy L. Davidson[2]

[1]*Sabanci University, Faculty of Engineering and Natural Sciences,Tuzla, 34956 Istanbul,Turkey*
[2]*Dept. of Electrical and Computer Engineering, Vanderbilt University, Nashville, TN, 37235, USA*

Abstract

In this paper, a new method to probe associative interactions of C-reactive protein (CRP) antigen with CRP antibody immobilized on a gold-interdigitated diamond electrodes was investigated. The CRP antigen detection was performed by capacitive/dielectric-constant measurements. Our results showed that 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. Biosensor developed in this study can be potentially used for detection of elevated CRP levels in suspected subjects for early diagnosis.

Keywords: Nanocrystalline diamond, Gold interdigitated, C-reactive protein, Biosensor.

1. Main text

C-reactive protein (CRP) is one of the plasma proteins known as acute- phase proteins and its levels 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[1-2]. CRP can rise as high as 1000-fold 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[1-3]. CRP was found to be the only marker of inflammation that independently predicts the risk of a heart attack[1,4]. Recently, different techniques have been reported to detect CRP antibodies and antigens. Meyer et al. developed magnetic biosensor and detected linear detection range from 25 µg L^{-1} to 2.5 mg L^{-1} for CRP antibodies[5]. A detection range of 5-25 mg L^{-1} was reported by Zhu et al. group[6]. These techniques are based on different approaches, and have lack of high sensitivity which is required when predicting the risk of coronary and cardiovascular diseases. Capacitance measurement could be a useful tool in immunoassay[7]. The attraction of affinity-based capacitive sensors are that they will be 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,

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

charge distribution, and conductivity change when an antibody-antigen complex formed on the surface of an electrode. In this study we are proposing an integrated solution for the detection and quantification of proteins to offer advantages of higher sensitivity, capability of single or multiple detection capability, easy to use, ease of signal processing with better sensor-signal integrity, smaller in size, compatibility to be integrated into a micro/electronics system, and much reduced system cost. Using CRP antibody as the model ligand/substrate, a direct detection of CRP by capacitance/dielectric measurements was demonstrated using a heterostructure of Au/nanocrystalline diamond covalently bound with CRP antibodies. When such CRP antibody immobilized heterostructure interacts with CRP antigen, the interaction of antibody with the antigen leads to the change in thickness of the dielectric layer and induces change in capacitance which can directly be related to detect the antigen.

2. Experiment details

2.1. Synthesis of nanocrystalline diamond film (NCD) and fabrication of gold interdigitated electrode

Nanodiamond films were formed with the process of the gases of $CH_4/H_2/N_2$ 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 on Silicon (1 0 0) substrate. Gold/interdigitated electrodes were fabricated on nanocrystalline diamond surface using image reversal technique. Length of each electrode was 750 µm with a width of 25 µm. The distance between two electrodes was 25 µm. A 40 µm deep SU8 wells were patterned over the interdigitated structure for easing the antibody immobilization on the sensor structure.

2.2. Materials

Monoclonal antibodies and purified antigen, C-reactive protein were 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.

2.3. Immobilization of C-reactive protein

For preparation of self-assembled monolayer (SAM), a clean gold interdigitated nanodiamond (GID-NCD) electrode was immediately immersed in a 10 mM mercaptopropionic acid (MPA) solution at room temperature for 24 h before being thoroughly rinsed with distilled water and dried over pure nitrogen gas. First, the carboxylic groups of SAMPA were activated by adding 0.05 M of EDC and 0.03M of NHS in phosphate buffer for 5 h. The amine activated GID-NCD electrode was incubated by adding 20 µl of 100 µg/ml CRP antibodies for 1 h at room temperature and then washed with PBS buffer followed by double distilled water. The free/unoccupied carboxyl groups on GID-NCD electrode surface was blocked by adding 100 mM ethanolamine buffer (pH 8.0) and incubated for 2 h at 4 ^{0}C followed by washing with PBS buffer and distilled water and finally dried. For CRP-antigen detection, a series of CRP-antigen concentration (0-1000 ng/ml) in 20 µl volumes was dropped on the electrode and incubated for 1 h.

2.4. Detection of CRP-antigen and characterizations

IR spectrum of surface activated GID-NCD was taken using a THERMO (Nicolet) 6700 Model FTIR spectrometer. Optical micrographs of the nanocrystalline diamond surfaces was studied. The degree of insulation of surfaces was characterized by cyclic voltammetry (BSA potentiostat system). Dielectric parameters (impedance/capacitance) were measured in the frequency range 50 MHz-1GHz 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 was calculated from measurements of the sample capacitance and extracted from the measurements at certain frequencies (f) of 50 to 400 MHz.

3. Results and discussion

3.1 FTIR spectra

To show the presence of amine groups on sensor surface, FTIR spectrum of the GID-NCD surface was recorded after the formation of SAM as shown in Fig. 1. The adsorption peaks in the range of 1750-1500 cm^{-1} are the deformation of vibrations of N-H bonding. 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. It was observed from the optical micrograph of GID-NCD surface that sputtered gold was homogenously well distributed on the surface.

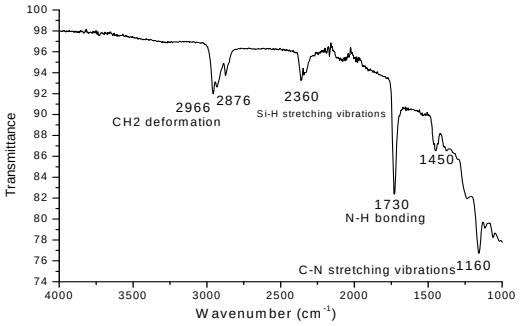

Fig. 1. FTIR spectrum of the sensor surface after the formation self assembled monolayer.

3.2 Electrochemical performance of the process of CRP antibody immobilization

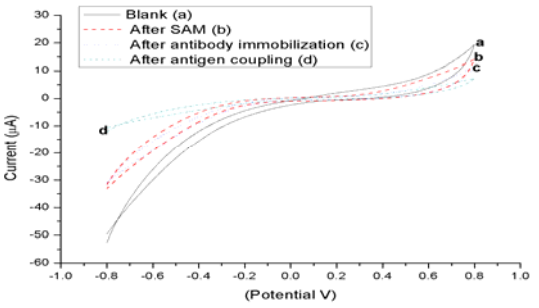

Fig. 2 Cyclic voltammograms of GID-NCD electrode obtained in a 5 mM $K_3[Fe(CN)_6]$ in PBS of pH 7.4 using : (a) blank electrode, (b) electrode modified with SAM, (c) immobilized with antibody, (d) coupling with antigen.

The degree of insulation was examined using cyclic voltammetry with a permeable redox couple $K_3[Fe(CN)_6]$ in the electrolyte solution. At the GID-NCD surface the redox couple was oxidized and reduced according curve Fig. 2a. When MPA was self-assembled on the clean GID-NCD surface the redox peaks decreased (curve b). Then antibody was reacted with the amine activated GID-NCD surface and antigen covalently coupled with CRP antigen. The insulating property of the electrode surface was further increased (curves c and d). This results suggested that the modified surface was well insulated and suitable for the capacitive systems[8].

Fig. 3(a-b) shows the variation of the dielectric constant as a function of the frequency and different concentrations of CRP antigen respectively. It was observed from the figure that dielectric constant passed through dielectric dispersion[9] and decreased with frequency. 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. The concentration and frequency above 800 ng/ml and 350 MHz, respectively showed no increase in response by this sensor system (Fig. 3b). This was possibly because of saturation of antibody

binding sites on the sensor surface. In addition, the sensor surface was bio-functionalized with a 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.

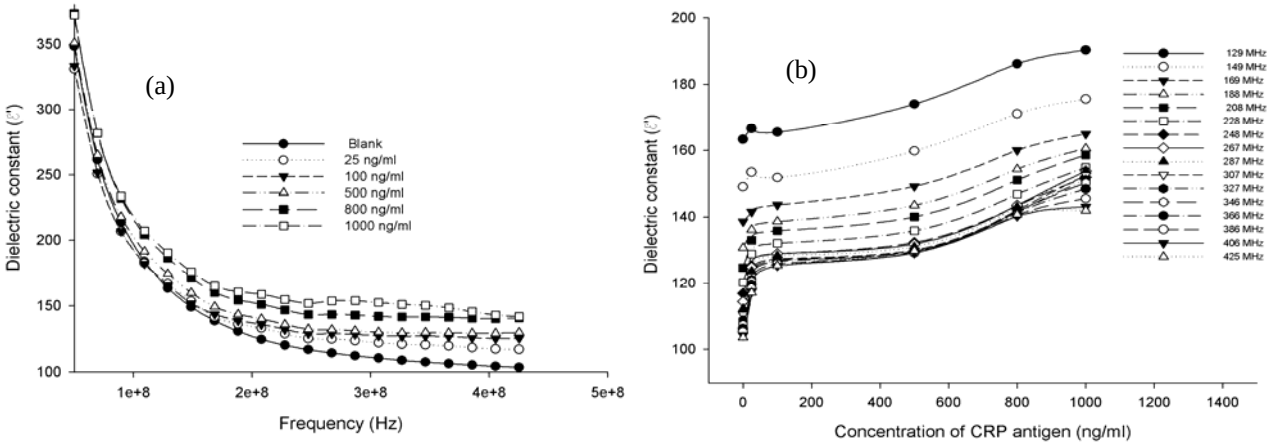

Fig. 3. The variation of the dielectric constant as a function of the frequency for the different concentrations of CRP antigen (a) and change in the dielectric constant with different concentrations of CRP antigen at different frequencies (b).

4. Conclusions

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 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.

Acknowledgments

We thank Bulent Koroglu for his 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.”

References

1. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. *J Clin Invest* 2003; 111:1805–12.

2. Lau DC, Dhillon B, Yan H, Szmitko PE, Verma S. Adipokines: molecular links between obesity and atheroslcerosis. *Am J Physiol Heart Circ Physiol* 2005; 288: H2031–41.

3. Casas JP, Shah T, Hingorani AD, Danesh J, Pepys MB. C-reactive protein and coronary heart disease: a critical review. *J Intern Med* 2008; 264:295–314.

4. Hansson GK. Inflammation, Atherosclerosis, and Coronary Artery Disease. *New England Journal of Medicine* 2005; 352:1685–1695.

5. Meyer MH, Hartmann M, Krause HJ, Blankenstein G, Mueller-Chorus B, Oster J, Miethe P, Keusgen M. CRP determination based on a novel magnetic biosensor. *Biosens Bioelectron* 2007; 22: 973–9.

6. Zhu Jun-Jie, Xu Jin-Zhong, He Jin-Tao, Wang Yi-Jun, Miao Qiang, Chen Hong-Yuan. An Electrochemical Immunosensor for assays of C-reactive protein. *Analytical Letters* 2003; 36:1547–1556.

7. Christine B, Bjarni B, Gillis J. Capacitive biosensors. *Electroanalysis* 2001; 13:173–80.

8. Warakorn L, Kanatharana P, Mattiasson B, Asawatreratanakul P, Thavarungkul P. A comparative study of capacitive immunosensors based on self-assembled monolayers formed from thiourea, thioctic acid, and 3-mercaptopropionic acid. *Biosens Bioelectron* 2006; 22: 233–40.

9. Grant EH. *Dielectric Properties of Biological Tissue and Cells in RF, an MW Frequencies*, New York: Plenum Press; 1983.