Sensors and Actuators B 209 (2015) 645–651

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Capacitive aptamer–antibody based sandwich assay for the detection of VEGF cancer biomarker in serum

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ARTICLE INFO

Article history:
Received 21 September 2014
Received in revised form 4 December 2014
Accepted 9 December 2014
Available online 16 December 2014

Keywords:
VEGF
Aptamer
Cancer biomarker
Biosensor
Capacitance

ABSTRACT

In this paper, we report on in vitro anti-VEGF aptamer selection and the development of a capacitive aptamer–antibody based sandwich assay for sensitive detection of vascular endothelial growth factor-165 (VEGF) in human serum. The assay design involves capturing of VEGF protein through two epitopes, one with anti-VEGF aptamer and the other with the antibody. The capacitive sensor was functionalized with anti-VEGF aptamer which first captures the VEGF protein followed by sandwiching with antibody conjugated magnetic beads (MB-Abs). Changes in capacitance was measured using non-Faradaic electrochemical impedance spectroscopy (nFIS) at different AC electrical frequencies (50–300 MHz). The sandwich assay format exhibited enhanced capacitive signal with respect to concentration with a detection range 5 pg mL^{-1} to 1 ng mL^{-1} of VEGF protein in human serum. Use of anti-VEGF aptamers enabled chemical stability on sensors during and after the chemical coupling on sensors. This work demonstrated the successful application of on-chip aptamer–antibody based sandwich assays for detection of target proteins in real serum sample for early cancer diagnosis.

1. Introduction

VEGF is a signaling protein, also used as a serum biomarker for a number of human diseases, including cancer [1,2], rheumatoid arthritis [3], psoriasis [4], and proliferating retinopathy [5]. VEGF potently promotes angiogenesis for vascular development, making it an attractive target for controlling angiogenic factor that plays a pivotal role in tumor growth and metastasis [6,7]. The abnormally fast growth and division of tumors prompts the over-expression of VEGF because of supply of more nutrients and oxygen, resulting in the induction of tumor lymphatic-vessels and the metastasis of cancer [8]. Patients with breast cancer have serum VEGF concentration range, from 18 to 328 pg/mL, whereas in healthy individuals, it is from 1 to 177 pg/mL, therefore an assay must be able to measure these concentration ranges [9]. Existing detecting methods such as immunohistochemistry (IHC) [10], enzyme-linked immunosorbent assays (ELISAs) [11] and fluorescence in situ hybridization (FISH) techniques [12] require sophisticated instrumentation that are complicated, expensive and time consuming. Therefore, it is imperative to develop a rapid, sensitive, label-free, and cost-effective method for the detection of VEGF in blood/serum from the suspected patients for early diagnosis.

Alternative new routes are ever demanding for developing suitable biosensors for disease diagnosis, such as by using synthetic biorecognition elements mainly ssDNA or RNA aptamers. Aptamers can effectively bind to their target biomarker proteins and offer specific properties of stability and resistance to denaturation, which favor them as new class of biorecognition elements for biosensors [13]. Aptamers can be selected through an in vitro selection process known as systematic evolution of ligands by exponential enrichment (SELEX) which yields synthetic nucleic acid ligands specific to desired target species from complex libraries [14]. The unique properties of aptamers enable designing innovative sensing protocols through interfacing them with electrochemical, optical or mass-sensitive transducing approaches [15–17].

Recently, several aptamer-based biosensors (aptasensors or aptamer beacons) [18] have been used for probing binding-induced conformational changes in aptamers and monitor their interaction with targets by means of color changes [19,20] or electron transfer [21,22]. However, these methods are limited by sensor size, sensitivity or complexity of assays. For example, colorimetric or fluorescence quenching methods require collecting a large amount of particles to induce a discernable color change or fluorescent signals. Whereas Faradiac principle based electrochemical sensing

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http://dx.doi.org/10.1016/j.snb.2014.12.040
0925-4005/

methods require redox mediating chemical to generate the signal and in some cases, long incubation time is required due to the slow diffusion of analyte through an unstirred layer to form the ligand–target complex [22] and these disadvantages as well as requirement of additional reagents limit such biosensors for application in disease diagnosis.

Capacitive based label-free biosensors offer one of the unique platforms that do not require mixing of sample with any additional reagent or redox mediator to generate the signal which is most desirable in cancer biomarker detection. A typical capacitive sensor utilizes non-Faradiac Impedance Spectroscopy (nFIS) principles to sensitively generate the signal in terms of dielectric properties, charge distribution depending upon the dimension of electrodes, when an analyte binds [23]. Immobilization of synthetic ligands such as aptamers on the surface of a capacitor sensor transducer provide adequate distance required for screening the surplus electrical charges by the mobile carrier redistribution [24]. Capacitance can then be measured by the amount of charge that a capacitor can hold at a given AC electrical frequency. Capacitance value can differ with the nature of molecules when introduced at the capacitor interface, for instance, charged molecule such as ssDNA aptamer or its target protein biomarker and the extent of signal may depend on size of a molecule. Similarly, the affinity with which the aptamer binds to its target on the sensor (electrode) surface also has a noticeable effect on the capacitance value [25]. The observed change in the capacitive signal can thus be used to quantify any reaction that occurred between the ligands immobilized on the metal surface and the target.

In this paper, a novel capacitive based apta-immunosensor was developed for VEGF detection in human serum which is simple, rapid and label-free in nature. The apta-immunosensor utilizes highly specific and selective anti-VEGF aptamer selected by the SELEX process which was functionalized on IDE capacitor surfaces (aptasensor). The signal generated with the developed aptasensor was easily enhanced by 3–8-folds by sandwiching VEGF antibody-coupled magnetic beads (apta-immunoassay). The proposed apta-immnoassay format provided most stable, sensitivity and selectivity platform for VEGF detection in spiked ten-time dilute human serum samples and the method reported in this study potentially finds application in clinical diagnosis.

2. Material and methods

2.1. Reagents

Human serum (male; blood type, AB) was purchased from PAN^{TM} Biotech, GmbH and reconstituted by appropriate dilution in phosphate buffered saline (PBS, pH 7.4) prior to use. 3-Mercaptopropionic acid (MPA), *N*-(3-dimethylaminopropyl)-N-ethyl carbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), human VEGF165 and monoclonal anti-VEGF antibody were purchased from Sigma–Aldrich, Germany. Surface activated MBs (Dynabeads M-270 Carboxylic Acid) were purchased from Life Technologies Co., USA. All other reagents used in this study were of analytical grade.

2.2. In vitro selection of anti-VEGF aptamer using SELEX technique

In vitro selection of anti-VEGF aptamer using modified SELEX technique and related experimental details are described in Supporting Information (SI) section 1.1.

2.3. Binding assays

One of the enriched sequences (anti-VEGF aptamer) after the SELEX process was selected which had a following sequence: 5′-GGG CCG TTC GAA CAC GAG CAT GGT GGG TGG TGG CCC TAG GAT GAC CTG AGT ACT GTC C-3′. Binding assay with anti-VEGF aptamer was performed to determine the aptamer's affinity and selectivity toward the target VEGF protein in binding buffer (BB) containing 100 mM NaCl, 20 mM Tris–HCl pH 7.6, 2 mM $MgCl_2$, 5 mM KCl, 1 mM $CaCl_2$, 0.02% Tween 20. The anti-VEGF aptamer sequence was commercially obtained after chemical synthesis with suitable modifications with fluorescently labeling at 5′ end of the sequence for further studies. Binding of anti-VEGF aptamer was carried out using varying amounts of VEGF-coated magnetic beads (corresponding to 0–1 μM VEGF protein) against constant 373 nM fluorescein labeled anti-VEGF aptamer. Fluorescently labeled aptamer enabled quantifying the fluorescence emission at 525 nm using a fluorospectrometer (Nanodrop, Thermo Scientific) to probe the changes occurred before and after binding of aptamers to the target protein. Relative fluorescence (%) was calculated using the following Eq. (1.1).

$$\frac{F - F_o}{F_o} \times 100 \tag{1.1}$$

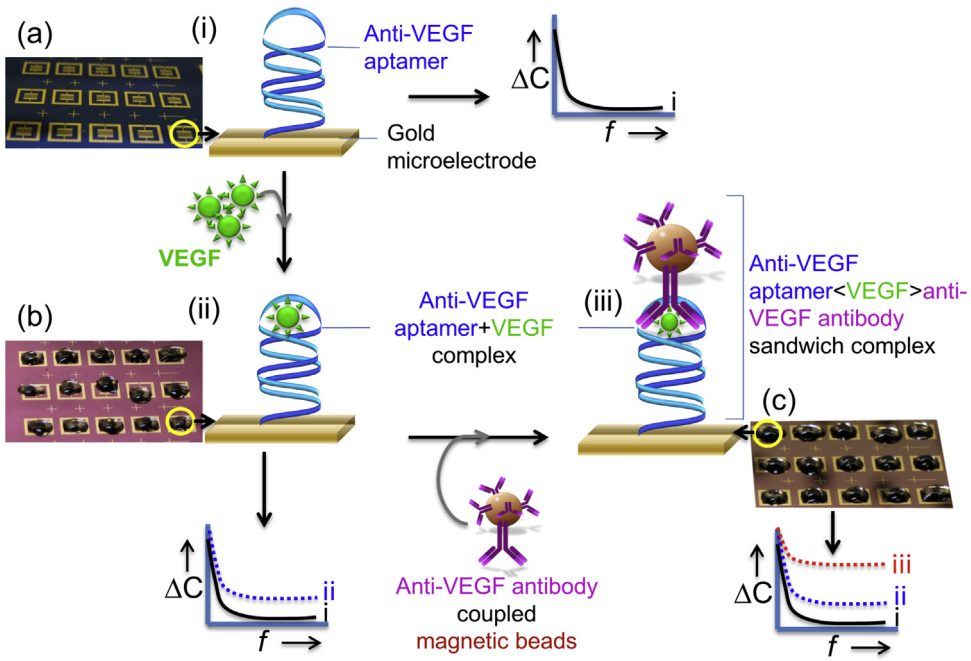
where F_o is the initial fluorescence and F is the fluorescence after binding. Here, various concentrations of VEGF protein coated magnetic beads were employed against constant fluorescein labeled anti-VEGF aptamers, respectively. The residual fluorescence derived from the aptamer was measured to calculate the amount of aptamer bound to the target protein. For testing specificity/selectivity of aptamers, instead of VEGF protein, a non-specific BSA protein coated magnetic beads were employed.

2.4. Capacitor sensor fabrication

IDE array based capacitors were patterned on a 525 ± 25 μm thick SiO_2 wafers (p-type, 0–100 Ω cm resistivity, ⟨1 0 0⟩ orientation) using standard photolithography. The wafer surface was cleaned in a series of steps using isopropanol, acetone and distilled water, respectively and dried using N_2 gas. Image reversal was carried out using AZ5214E photoresist after layering it on SiO_2 wafers and baked at 120 °C for 5 min. Following this step, a 50–60 nm thin tungsten layer was deposited to facilitate improved adhesion of gold and a 200–210 nm thick gold layer was then deposited using direct current sputter deposition. The deposition was carried out in argon atmosphere with power of 150 W for 3 min. The gold was lifted off using acetone and the dimension of each electrode was measured to be 800 μm in length and 40 μm in width with a distance between two electrodes of 40 μm. Each wafer contained an array of 45 independent capacitors each made of 24 IDEs within a total area of 3 mm^2 that served as individual sensors.

2.5. Apta-immunosensor development and signal measurement

A label-free capacitance based apta-immunosensor was designed and fabricated as described in experimental methods (Scheme 1). Gold IDE capacitors were fabricated on SiO_2 wafers in multiple arrays in which each capacitor was made of 24 gold IDE microelectrodes measuring a sensing area of 3 mm^2 which was designed to hold ~5 μL sample volumes. Here, "aptasensor" was termed for the capacitor sensor functionalized with anti-VEGF aptamer. The "apta-immunosensor" was termed for the aptasensor that was first allowed to capture VEGF protein and later sandwiched with MB-Abs for enhanced signal-to-noise ratio and the stepwise details of experimental methods are described below.



Scheme 1. Schematic illustration of apta-immunosensor showing changes in capacitance against the applied AC electrical frequency: (a) sensor surfaces functionalized with aptamers (aptasensor) as blank surfaces; (b) aptasensor incubated with 0.1× serum containing spiked VEGF protein at different concentrations and (c) sandwiching of aptasensor with MB-Abs on aptamer–VEGF protein complex fromed in step (b). The images shown in (i), (ii) and (iii) are real images of capacitor arrays taken at different steps.

2.5.1. Immobilization of anti-VEGF aptamer on IDEs of capacitor chips (sensors)

The IDE capacitor chips were first immersed in a solution of 100 mM of β-mercaptopropionic acid (MPA) in ethanol for 12 h at room temperature, washed with ethanol and dried using N_2 gas. Self-assembled monolayer (SAM) of MPA containing free carboxyl groups on sensors were activated by adding a mixture of 200 mM of EDC and 100 mM of NHS in distilled water and incubated for 3 h. The activated sensor chip made of arrays of capacitors each with an area of 3 mm^2 were incubated with 2 μM of 5′-NH_2-$(CH_2)_6$-modified anti-VEGF aptamer (ssDNAs) in 5 μL volumes for 3 h and washed with PBS, pH 7.4 and pre-incubated at 37 °C for 1 h in an air-tight humid chamber. The unprotected groups on aptamer functionalized sensors were capped first by incubating for 15 min with 50 mM ethanolamine in PBS, pH 8 and washed and stored at 4 °C until use.

2.5.2. Pre-treatment of immobilized anti-VEGF aptamers on IDEs

The aptamer immobilized sensors were subjected to pre-treatment just before their use, to avoid undesirable aptamer folding that may prevent from interactions with their targets. Therefore, pre-treatment of sensors were done first by placing the chips in a pre-heated airtight moist chamber at 90 °C for 10 min, quickly cooled at 4 °C and finally incubated at 25 °C for 10 min followed by incubating the sensors in a blocking solution containing 1% BSA in PBS, pH 7.4 for 1 h. Preparation of VEGF protein spiked human serum sample is described in SI section 1.2. A series of VEGF protein concentrations in 5 μL 0.1× human serum ranging 5 pg mL^{-1} to 5 ng mL^{-1} were incubated on aptasensors, each covering an area of 3 mm^2 IDEs and incubated for 2 h at room temperature. The resulting complex of aptamer + VEGF antigen formed on sensors was designated as a primary complex.

2.5.3. Conjugation of antibodies on magnetic beads (MBs)

Surface activated MBs were conjugated with VEGF protein specific monoclonal antibodies using carbodiimide coupling as per the manufacturer's instructions. Briefly, 2×10^8 MBs in 100 μL volume were washed with 25 mM MES buffer, pH 5 and suspended in a mixture of 1-ethyl-3-(-3-dimethylaminopropyl) carbodiimide (EDC) solution (50 μL, 50 mg mL^{-1}) and N-hydroxysuccinimide (NHS) solution (50 μL, 50 mg mL^{-1}) and incubated for 30 min at room temperature under shaking conditions. The MBs were washed twice with MES buffer and incubated with 100 μL of 2.7 μg of anti-VEGF antibody solution in same buffer. The MBs were magnetically separated and washed twice with the same buffer. Thus obtained antibody coated MBs (MB-Abs) were suspended in 100 μL of 50 mM ethanolamine in PBS pH 8 and incubated at room temperature under shaking conditions for 1 h and magnetically separated that enabled blocking of unreacted carboxyl groups on MBs. Thus obtained MB-Abs were washed thrice with PBS, pH 8 containing 0.05% tween 80 and resuspended in 100 μL of PBS pH 7.4.

2.5.4. Sandwiching of MB-Abs on aptasensors (apta-immunosensor) for signal enhancement

Sandwich assay was carried out using a constant 7.5×10^6 MB-Abs suspension in 5 μL PBS, pH 7.4 and incubated for 1 h on each IDE capacitor that previously contained aptamer captured with VEGF antigen (primary complex). The resulting sandwiched complex of aptamer + VEGF + antibody + MBs was termed as a secondary complex (apta-immunosensor). The sensors were then washed thrice with PBS and dried gently using a N_2 stream. For negative controls, bovine serum albumin (BSA) at different concentrations were spiked in human serum and used as negative controls under standard assay conditions. Bare IDE capacitors without aptamers functionalized were also tested by incubating with MB-Abs for determining any non-specific binding under identical conditions. Optical images of the sensors before and after sandwiching with MB-Abs were taken using Carl Zeiss Axio Scope A1 MAT.

2.5.5. Dielectric measurements

Changes in dielectric parameters (impedance/capacitance) in between the gold interdigitated microelectrodes of capacitors before and after incubation of samples on the sensor surfaces was measured in the frequency range 50 MHz to 300 MHz using

a Network Analyzer (Karl Süss PM-5 RF Probe Station and Agilent-8720ES), which was pre-calibrated using SOLT (short-open-load-through) method. The impedance values were exported to Matlab® software for the analysis and the capacitance values were extracted from triplicate experiments at an effective frequency (f) range between 50 and 300 MHz. The results were normalized with respect to blank controls the final response values of independent replicates ($n = 3$) were averaged, plotted and the standard deviations were shown as error bars. Percent relative standard deviation (%RSD) was calculated from the replicate experiments that were found to be less than ~12%.

3. Results and discussion

VEGF is a relatively small protein with a molecular mass of 38.2 kDa and label-free biosensing of a small protein is a challenging process. Here, we designed a unique sandwich assay based on sequential steps involving (a) in vitro selection of ssDNA aptamers that specifically bind VEGF protein using SELEX technique, (b) fabrication of gold IDE capacitor arrays, (b) immobilization of anti-VEGF aptamer on IDE capacitors as specific recognition elements, (c) initial capturing of immobilized anti-VEGF aptamer with VEGF protein on sensors (aptasensor) and (b) sandwiching MB-Abs on aptasensors for specific enhancement of the capacitive signal (apta-immunosensor). While all the reactions taking place on the capacitive sensor, capacitance responses were recorded to probe the real-time changes in the capacitive signal. In this study, we employed ssDNA aptamers in place of antibodies initially to immobilize on sensors because antibodies often are susceptible to inactivation or irreversible denaturation during the chemical coupling processes mainly on planar IDE capacitor surfaces. Therefore, a series of steps as illustrated in Scheme 1, a sensitive label-free detection of VEGF protein in human serum was designed and the results of this assay are described in following sections.

3.1. In vitro selection of anti-VEGF aptamer

A highly specific anti-VEGF ssDNA aptamer was first selected using a modified SELEX technique as described in the experimental methods. This aptamer was evolved from a random library of 10^{15} ssDNA molecules through successive 12 iterations that yielded a highly specific anti-VEGF aptamer. Binding of anti-VEGF aptamer was further confirmed by incubating varying VEGF protien-coated magnetic beads (corresponding to 0–1 μM VEGF) against constant 373 nM fluorescein labeled anti-VEGF aptamer. Fig. 1 shows an increase in % relative fluorescence with increase in the VEGF protein concentrations until reaching to its saturation. Nonlinear analysis by fitting the binding data of anti-VEGF aptamer and VEGF protein showed the dissociation constant (K_d) of 315 nM (Fig. 1, inset). As expected, the anti-VEGF aptamer, however failed to bind BSA, a non-specific protein under standard assay conditions indicating its specificity to VEGF protein (Fig. 1). The specificity of anti-VEGF aptamer to VEGF protein was originated after extensive negative selection steps introduced during the SELEX process (see SI section 1.1.2). This aptamer was employed as a stable biorecognition element for the development of aptasensor. Here, use of ssDNA aptamer in biosensing provided greater stability because of their resistance to denaturation during the chemical coupling and sensor washing procedures compared to antibodies [26].

3.2. VEGF protein detection using apta-immunosensor

The anti-VEGF (ssDNA) aptamer selected in vitro was first 5′-NH_2-$(CH_2)_6$-modified and immobilized on SAM modified gold IDE capacitors. A series of VEGF protein spiked in 1:10 diluted

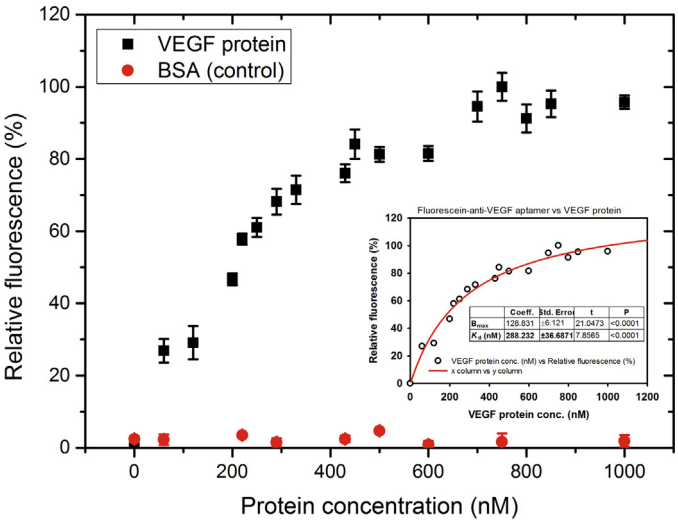


Fig. 1. Binding of free 5′ fluorescein labeled anti-VEGF aptamer with VEGF protein coupled on MBs. Measured changes in relative fluorescence (%) values against varying VEGF protein cocentrations of VEGF protein and BSA shown in back and red, respectively. The inset figure shows non-linear fit curve using the relative fluorescence data plotted under one-site binding mode.

human serum was incubated on an array of capacitors each measured an area of 3 mm^2 as described in experimental methods. Changes in capacitance signal were recorded that was generated as a result of interaction of aptamer and VEGF protein from spiked human serum on aptasensor. Capacitor IDEs immobilized with anti-VEGF aptamers transformed them into abundant negatively charged surfaces because of the ssDNA's phosphodiester backbone and thus, reduced surface capacitance [21]. A small change in capacitance signal was observed as the anti-VEGF aptamers captured the VEGF protein at the interface of the sensors. Fig. S1a shows changes in capacitance responses of aptasensors incubated with varying concentrations of VEGF protein at different frequencies from 50 to 300 MHz. The small capacitive changes can be attributed to the smaller size of VEGF protein and thus less surface charges distributed against large negatively charged ssDNA backbone (aptamer) which contributed to a small change in surface capacitance (Fig. S1a). However, there was no response at all with BSA protein under identical conditions (Fig. S1a, inset). A maximum of only about 3–15% relative capacitance change was occurred with the aptamer and VEGF complex (Fig. S1b).

Studies have shown that a large capacitance enhancement can be induced by metal–doping on electrical double layer capacitors [27]. The aptasensor signal can therefore be enhanced through similar metal-doping or functionalization mechanisms to induce the capacitance signal. The above principle was tested in this study on aptasensors after subjecting to specific deposition of MB-Abs on aptasensor which previously contained aptamer–VEGF protein complex, thereby, no non-specific deposition would occur in the absence of VEGF protein. The MBs used in this study were made of iron oxide magnetic particles precipitated with cross-linked polysterene polymer, which was conjugated with antibodies specific to VEGF protein. The iron oxide in MB-Abs also contributed to the enhanced capacitance signal. Sandwiching of aptamer–VEGF complex formed on aptasensors was therefore carried out by incubating the aptamer–VEGF complex on aptasesnor with constant amount of MB-Abs (7.5×10^6) carrying VEGF-specific-antibodies (Scheme 1). The optical images of sensors after the MB-Abs sandwiching were acquired that clearly showed VEGF specific capturing of MB-Abs occurred on sensors (Fig. 2a–d).

The results showed a significant enhancement in responses in a dose-dependent manner. Fig. 3a shows capacitance responses

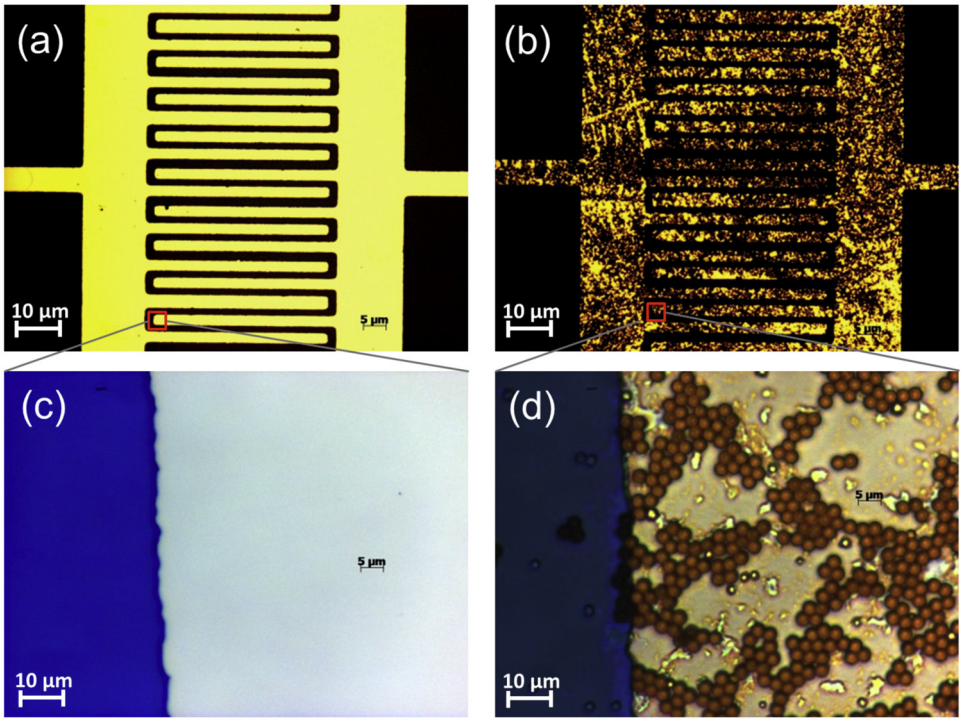


Fig. 2. Optical microscopic images of sensor surface: (a) control and (b) after sandwiching with MB-Abs (magnetic beads functionalized with VEGF antibodies). Images shown in (c) and (d) are enlarged view of a portion from (a) and (b), respectively that were taken at higher resolutions (100×).

of apta-immunosensor after incubation with VEGF protein specific MB-Abs under the applied AC electrical frequency. It is clear from this result that a greater resolution in signal responses with respect to varying target concentrations was observed after MB-Abs sandwiching as compared to that in absence of MB-Abs (Fig. S1a and S1b). Fig. 3b shows dose-dependent capacitance responses extracted at 65, 90, 120 and 212 MHz frequencies that exhibited a dynamic detection range from 5 to 1000 pg mL^{-1} which is comparable to previously reported studies using the most sensitive surface plasmon resonance (SPR) analysis [18]. A negative control, BSA protein and a bare IDE surface (in absence of aptamer) was used to test the specificity of the sensor responses under identical conditions that did not show significant change in capacitance (Figs. 3a and S1, inset).

Sandwiching MB-Abs on aptasensors improved the performance of the capacitor sensor because, antibodies on beads were specifically interacted with VEGF protein which was previously captured by anti-VEGF aptamers on sensor, and thus 3–8-folds enhancement in the signal was evident (Fig. 3). Extrapolated limit of detection (LOD) from the regression analysis of sensor responses showed its strong relationship with the applied AC frequency. Minimum-LOD was 401 pg mL^{-1} at 65 MHz and 439, 550 and 813 pg mL^{-1} VEGF protein at 90, 120 and 212 MHz frequencies, respectively (Figs. 3b; 4a and b and 5). We found that the MB-Abs sandwiching enabled signal enhancement even with small changes in VEGF concentrations in dilute serum compared with only aptamer VEGF complex (Fig. 4a and b). The aptamer alone in case of aptasensor probably tend to partially unfold which may lead to suppressing the surface charge distribution between the electrodes and therefore, small capacitance changes was seen without sandwiching [21]. Capacitive responses of the presented sensors are mainly derived from the insulating layers on the

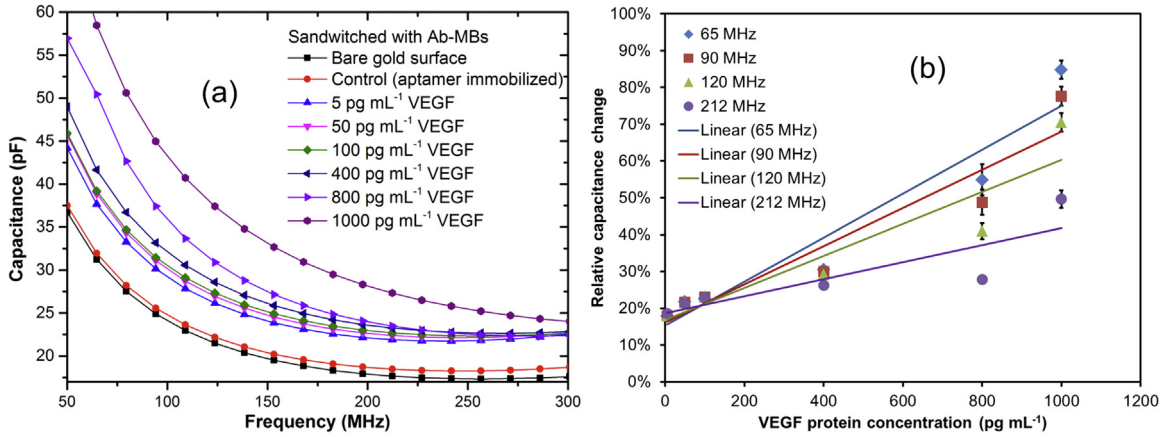


Fig. 3. (a) Capacitance response profiles as a function of applied AC frequency with the developed apta-immunosensor upon sandwiching with constant amounts of monoclonal antibodies for VEGF protein coated on MB-Abs on the aptamer–VEGF protein complex previously formed on the sensors. (b) Percent relative change in capacitance responses of apta-immunosensor extracted at four different frequencies indicated in figure legend and the data were fitted with linear regression analysis.

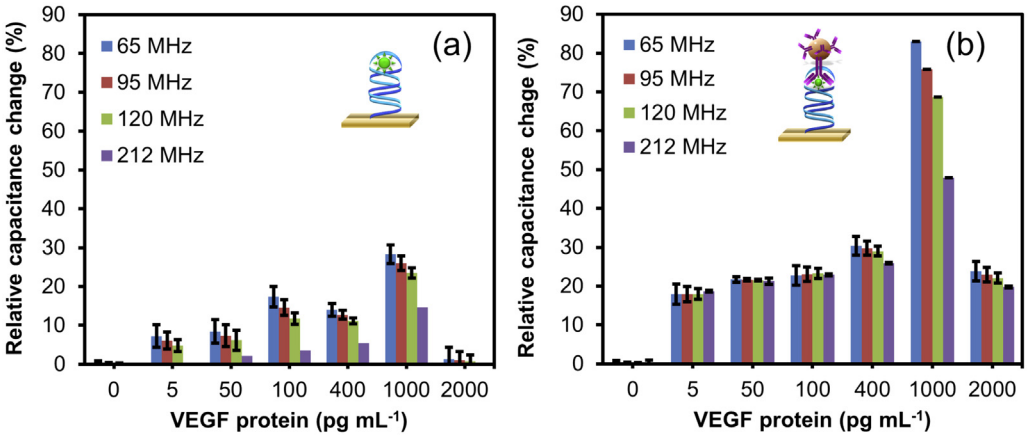


Fig. 4. Relative percent changes in capacitance responses occurred at different frequencies (65, 95, 120 and 212 MHz) with; (a) primary complex (aptasensor, before sandwiching) and (b) secondary complex (apta-immunosensor, after sandwiching with MB-Abs) formed on the sensor surfaces. The insets in figures (a) and (b) represents illustrations before and after sandwiching of aptamer–VEGF protein complex with MB-Abs.

electrodes combined with the conductance, originating from the charge redistribution and recombination within these layers on sensors imparted by the aptamer-protein interactions.

The incompact arrangement of MB-Abs on sensors can also provide sufficient area to obtain favorable orientations to interact with targets in close proximity on the electrode interfaces. Also, binding of MB-Abs tend to decrease the distance between the electrodes, which may have also contributed to enhancing the capacitive signal. As a result, the MB-Abs sandwiching assay yielded larger change in capacitance responses and thus enabling the LOD of a several hundred picograms of VEGF protein from dilute human serum at varying AC frequencies such as 65, 95, 120 and 212 MHz (Figs. 3b; 4a and b and 5). However, the apta-immunosensor had a limitation in sensitivity from 5 to 1000 pg mL^{-1} VEGF protein probably due to the limited availability of VEGF protein molecules on a given sensor area (3 mm^2). At highest concentration (2000 pg mL^{-1} VEGF protein), the sensor signal dramatically decreased. We speculate that this could be because of the strict competition between the abundant target molecules (VEGF protein) and limited local aptamer binding sites. This type of competition likely to alter the local/proximal physico-chemical environment and therefore dissociation of previously captured target molecule. However, the detection range can also be fine-tuned by optimizing the sensor area, geometry and sizes of MBs for obtaining a desired detection range.

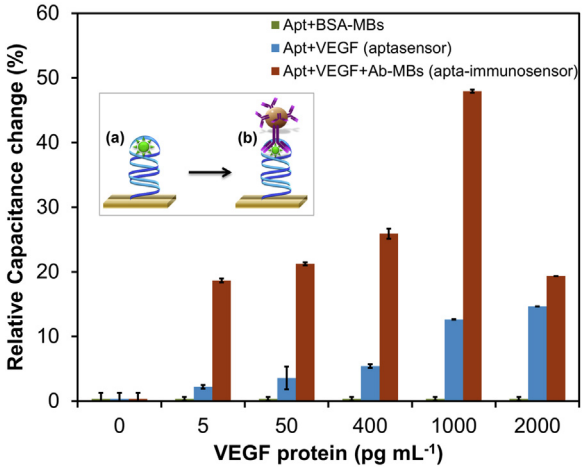


Fig. 5. Comparitive relative percent change in capacitance responses at 212 MHz with primary complex (aptasensor) and secondary complex (apta-immunosensor) formed on sensor surfaces. The inset figures (a) and (b) illustrates the formation of complex on a section of gold IDE surface before and after the signal enhancement, respectively.

4. Conclusions

A simple and sensitive approach for VEGF detection was reported which was based on label-free capacitive sensor platform that utilizes anti-VEGF aptamers to first capture serum VEGF protein and exhibit small change in capacitance (aptasensor). This signal was further enhanced by 3–8-folds through sandwiching MB-Abs (apta-immunoassay). Interaction of MB-Abs with VEGF protein-anti-VEGF aptamer complex on capacitor surface contributed to the formation of an additional layer on sensors and therefore, enhanced the capacitance performance and improved the sensitivity. VEGF protein specific capturing of MB-Abs on sensors also provided incompact arrangement of MB-Abs on sensors, which facilitated reduction in the distance between the electrode, an essential feature of enhancing the capacitive signal of a typical IDE capacitor. This method also enabled prevention of undesirable interactions between the aptamer probes and non-specific proteins that usually accompany with the targets in serum environment, resulting in significant increase in specific charge distribution efficiency on sensor surface. The developed apta-immunosensor/assay showed a dynamic detection range from 5 pg mL^{-1} to 1 ng mL^{-1} of VEGF protein in human serum with no additional reagent or redox-chemical required for detection of VEGF protein. Therefore, the developed biosensing approach can provide a convenient and sensitive means to recognize target molecules in real sample that is most desirable in clinical diagnosis. However, considering the fact that detection of lone biomarker may not always be accurate for the diagnosis of a disease. Therefore, our future experiments are aimed at utilizing the similar sensor platform for detecting multiple biomarkers that are associated with cancer risks which can then potentially allow accurate diagnosis of this disease.

Acknowledgements

This work was supported by the Scientific and Technological Research Council of Turkey (TUBITAK), Grant no: 110E287. We thank Irena Roci and Divya Mahalingam for their assistance with in vitro aptamer selection and chip fabrication, respectively.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.snb.2014.12.040.

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