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Rapid Detection of Protein Kinase on Capacitive Sensing Platforms

Rohit Chand, Dawoon Han, and Yong-Sang Kim

***Abstract*— In this study, we developed a capacitive sensor for the one-step and label-free detection of protein kinase A (PKA) enzyme. Metal-insulator-semiconductor (MIS) and electrolyte-insulator-semiconductor (EIS) are a simple electronic transducer, which allows efficient detection of the target analyte. For this reason, we performed a comparative sensing of PKA on the MIS and EIS capacitive sensor. The PKA-specific aptamer was used for the one-step detection. For the immobilization of thiolated aptamer, the MIS sensor contained a thin gold layer, whereas the EIS sensor had a self-aligned monolayer of gold nanoparticles. The interaction of aptamer and PKA changed the charge and density of the sensor surface. The quantitative detection of PKA was performed by analyzing the capacitance-voltage curve after the aptamer-PKA interaction. The MIS and EIS sensor showed a detection limit of 5 U/mL and 1 U/mL, respectively, for the detection of PKA. This study suggests valuable sensing platforms for the rapid and sensitive biochemical diagnosis.**

***Index Terms*— Biosensor, Aptamer, Capacitance, Protein kinase A, Electrolyte-insulator-semiconductor, Metal-insulator-semiconductor,**

I. Introduction

Regulation of protein function through phosphorylation is an important aspect of post-translational modification. Protein kinases are enzymes that catalyze the addition of a phosphate group to target proteins, thus controlling the cellular life. Many naturally occurring toxins and pathogens exert their effects by altering the phosphorylation of intracellular proteins. Abnormal phosphorylation of protein can lead to several medical conditions, including cancers, Alzheimer's, and auto-immune diseases [1, 2]. Protein kinases are an important drug target due to their critical function in signal transduction pathways and post-translational modifications. Therefore, rapid and effortless quantification of the protein kinase is crucial for the biochemical and medical diagnosis.

In this respect, mass spectroscopy, radioisotope labeling, or enzyme-linked immunosorbent assays based kinase detection are routinely employed [3,4]. These techniques present several limitations and typically require half to a full day for analysis. Traditional assays consume many expensive and hazardous reagents and require a number of specialized and often expensive instruments. Additionally, miniaturization of these techniques for the point-of-care use is quite not possible. Recently, electrochemical and optical based detection has proved to be a sensitive and selective approach [5,6]. However, most of the recent methodologies depend on the kinase-catalyzed phosphorylation of peptide. In addition, they require tagging of the electroactive molecule like nanoparticle or fluorescent probe, to the adenosine triphosphate or the enzyme substrate. Therefore, multi-step detection of a kinase enzyme, involving washing, incubation, reaction, and detection sequence make it time-consuming, expensive, and laborious. Furthermore, the use of numerous biomolecules for recognizing and reporting reduces the commercial applicability of these protocols.

For these reasons, in this work, we designed a one-step aptameric biosensor based on capacitive detection platform. Electrolyte-insulator-semiconductor (EIS) and metal-insulator-semiconductor (MIS) are simple ion-sensitive field-effect transistors with capacitive detection [7]. They detect analyte through a direct change in the gate voltage or capacitance resulting due to the biomolecular interactions [8,9]. Electronic transducers are easy to fabricate at a low-cost, sensitive, label-free, and can be miniaturized easily. A typical EIS or MIS sensor relies on the release of protons (change in local pH) during the biochemical reaction, which changes the space-charge of the semiconductor-insulator interface leading to a shift in the gate voltage [8]. More recently, a number of capacitive sensors have been reported which rely on the intrinsic charge of the biomolecule and their interaction, rather than the release of protons [7,10]. The majority of biomolecules have certain charge under physiological conditions. The intrinsic charge can also be increased or the polarity can be reversed in-situ by focusing on the isoelectric point (pI) of the biomolecule. The change in the local surface charge modulates the electrical properties of EIS or MIS capacitors. Such capacitive sensors are easier to operate and open up possibilities to detect several analytes.

The surface of the EIS or MIS sensor can be modified with receptor molecules to make it selective for the target molecule and to prevent nonspecific interaction [10,11]. Aptamers are oligonucleotide or peptide, analogous to antibodies, which bind to a target molecule with high specificity and affinity. In addition, it has been widely demonstrated that the interaction of aptamer with the target changes the structure of the aptamer and total charge and charge distribution of the complex [12,13].

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Therefore, the capacitive sensor modified with the kinase-specific aptamer can be used for the detection of kinase enzymes, without depending on the kinase-catalyzed phosphorylation and labeling of the molecules.

In this study, we developed a label-free one-step detection of protein kinase A (PKA) using PKA-specific aptameric peptide on the capacitive sensing platform. Silicon dioxide (SiO_2) grown on the p-type silicon (Si) was used as the substrate for the sensor. The detection was based on the change in local surface charge due to aptamer-PKA interaction. We compared the working of MIS and EIS for the detection of PKA. A thin gold layer was deposited on the gate insulator for the MIS based sensing. Whereas, gold nanoparticles were attached to the gate insulator for the EIS based sensing. Scanning electron microscopy was used to study the surface modification of EIS sensor. Capacitance-voltage curves were recorded to detect the presence of PKA. As a proof of concept, we also detected PKA in the spiked human cell sample.

II. Experimental

A. Materials

cAMP–dependent protein kinase A and protein kinase buffer was purchased from New England Biolabs (USA) and stored at -20 ℃. Hydrogen tetrachloroaurate(III) hydrate ($HAuCl_4 \cdot 3H_2O$), sodium citrate, 3-Mercaptopropyl)trimethoxysilane (MPTS), tris(hydroxymethyl)aminomethane (Tris), sodium chloride, and hydrochloric acid of highest purity were purchased from Sigma-Aldrich (USA). Thiolated aptameric peptide (Mpr-TTYADFIASGRTGRRNAIHD) was obtained from AnyGen co. Ltd (Korea) [14]. All other reagents were of analytical grade and purchased from Sigma-Aldrich. De-ionized (DI) water was used throughout the experiment.

B. Synthesis of gold nanoparticles

The AuNPs (d ≈ 16 nm) were synthesized using a seedless method as described before [15]. Briefly, 20 mL of 1.0 mM aqueous $HAuCl_4 \cdot 3H_2O$ solution was first brought to a boil. Next, 2 mL of 38.8 mM aqueous solution of sodium citrate was added, which was then boiled for 10 min until the color changed to deep red. The synthesized particles were characterized using UV-vis spectroscopy and scanning electrode microscope.

C. Fabrication of capacitive sensors

p-doped silicon substrate with a resistivity of 10 Ω-cm was used for the fabrication of sensors. Before the deposition of SiO_2 film, the Si wafer was cleaned using a standard Radio Corporation of America (RCA) process. A 50 nm thick SiO_2 was grown on the Si substrate through plasma-enhanced chemical vapor deposition. Next, the back of Si wafer was primed using the wafer back grinding process to obtain a 200 µm thick substrate. After priming, 100 nm thick aluminum (Al) was thermally deposited on the back of Si wafer using a vacuum thermal evaporator to serve as a back-contact. In the case of MIS sensor, additional 100 nm thick gold (Au) layer with titanium as a supporting layer was deposited on the SiO_2 surface. The 3M™ double-sided tape was pasted on MIS and EIS sensors after the surface functionalization (section II.D) [1]. The tape formed a reservoir with an active area of 5 mm × 5 mm. Finally, the sensors were bonded on the Al coated glass surface, which aids in the back-contact of the sensor.

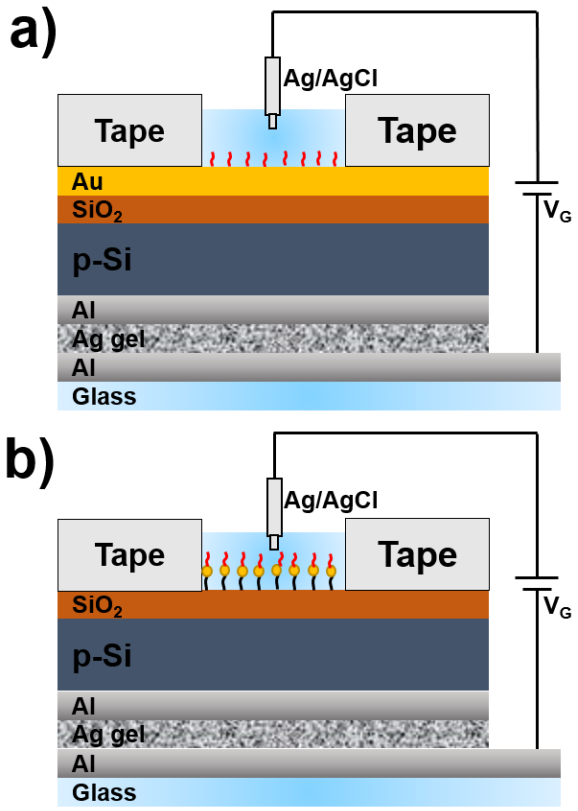

Fig. 1. Structure of capacitive MIS sensor (a) and EIS sensor (b).

D. Surface functionalization of the sensors

The gold thin layer containing MIS sensors were directly functionalized with the aptamer. A 100 µM solution of aptamer was prepared in tris buffer saline (2 mM tris, 20 mM NaCl, pH 7) and dropped on the clean MIS surface. The aptamer was allowed to immobilize for 6 hours at 4 ℃ that formed a self-aligned monolayer. The surface was rinsed with DI water before the PKA interaction.

The functionalization EIS sensor was initiated by first treating the SiO_2 surface with O_2 plasma for 5 min at 50 sccm flow rate and 5×10^{-2} Torr pressure. The O_2 plasma treatment activated the hydroxyl groups on the SiO_2 surface, which is beneficial for the reaction with silane molecule. Silanization of the SiO_2 surface was performed by dipping the EIS sensor in 1% MPTS-toluene solution for 3 hours. A self-aligned monolayer was formed by the reaction between the hydroxyl group of the EIS surface and the silane group of the MPTS, leaving free thiol group on the top. The silanized surface was thoroughly rinsed with the toluene followed by the ethanol. To strengthen the silane bonds and activate thiol groups, the EIS sensor was heated at 110 ℃ for 15 minutes [16]. Next, AuNPs were attached on the MPTS modified EIS sensor for 6 hours. The reaction between thiol groups of the MPTS and Au captured the AuNPs on the EIS sensor. The AuNPs modified

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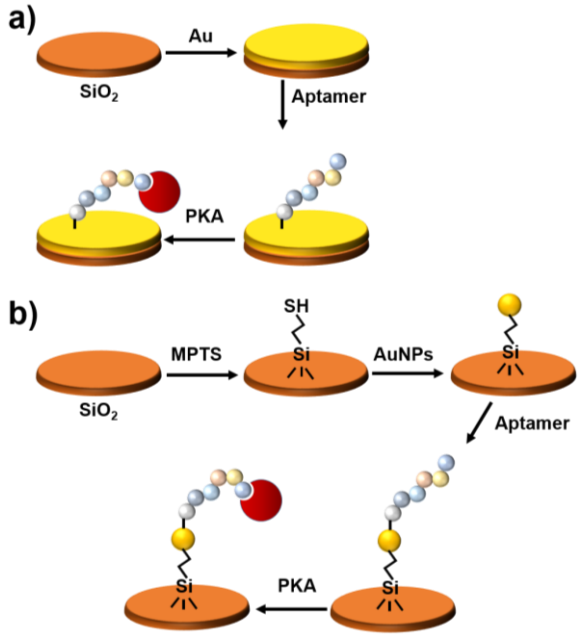

Fig. 2. Scheme of aptamer based PKA detection on MIS sensor (a) and EIS sensor (b).

EIS sensor was characterized by the scanning electron microscopy (SEM). The functionalization of the EIS sensor was finalized after immobilizing the 100 µM aptamer solution on the AuNPs for 6 hours at 4 ℃. The aptamer terminated MIS and EIS sensor were further used for the detection of PKA.

E. Protein kinase A detection on capacitive sensors

The one-step detection of PKA was performed by adding 10 µL of different concentrations of PKA in 1X PKA buffer on the aptamer functionalized capacitive sensors. The PKA was allowed to interact with the aptamer for 20 minutes. After the interaction, the reservoir was slowly filled with tris buffer for the capacitive analysis. Capacitance–voltage (C–V) analysis of the capacitive sensors was performed to detect the PKA concentration. C–V measurements were performed using a Hewlett-Packard (HP) 4284A LCR meter and Ag/AgCl reference electrode. The gate voltage (V_G) was swept with a superimposed AC signal of 10 mV. The MIS sensor was operated at a frequency of 5000 Hz, whereas the EIS sensing was performed at 1000 Hz.

F. Detection of PKA in cell sample

Human prostate cancer cell line LNCaP was obtained from American Type Culture Collection (USA). Cells were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum at 37℃ in a humidified 5% CO_2 incubator. Cells from subcultures were supplemented with 0.01% trypsin-EDTA (Sigma-Aldrich, USA). Finally, the cells were spiked with the reaction mixture (1X PKA buffer) containing different concentrations of PKA. For the PKA detection, 10 µL of spiked cell sample was incubated with the aptamer-modified MIS sensor and then analyzed as described earlier.

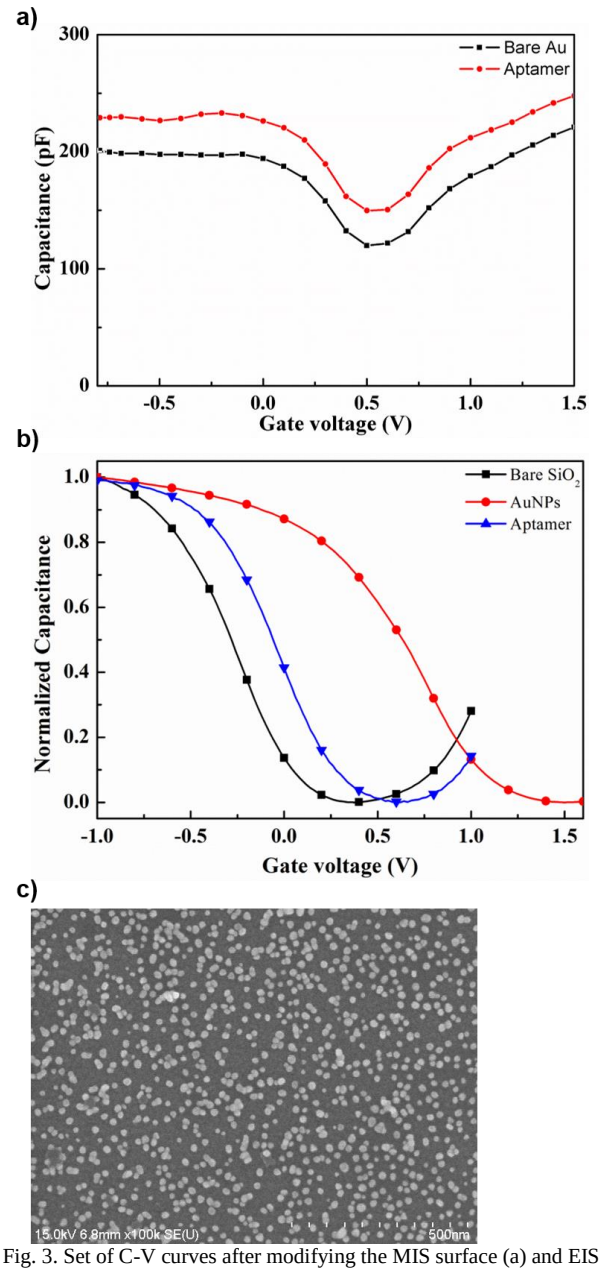

Fig. 3. Set of C-V curves after modifying the MIS surface (a) and EIS surface (b) with 100 µM aptamer for 6 hours. Buffer: 2 mM Tris-Cl saline, pH 7. (c) SEM image of the EIS surface modified with AuNPs.

III. Results and Discussion

A. Characterization of sensors

The structure of the fabricated MIS sensor (Al–Si–SiO_2–Au) is shown in Fig. 1(a). The tape acted as a reservoir for storing the analyte and buffer during analysis, whereas the Al helped in back-contact of the sensor. The scheme for PKA analysis using MIS sensor is summarized in Fig. 2(a). In summary, the monolayer of aptamer formed binding sites for the specific detection of PKA. Next, different concentrations of PKA solution in 1X reaction buffer was dropped on the sensor surface for interaction with the aptamer.

The structure of the fabricated EIS sensor (Al–Si–SiO_2–AuNPs) is shown in Fig. 1(b). In the case of EIS sensor, citrate-capped AuNPs were attached on the bare SiO_2 surface using

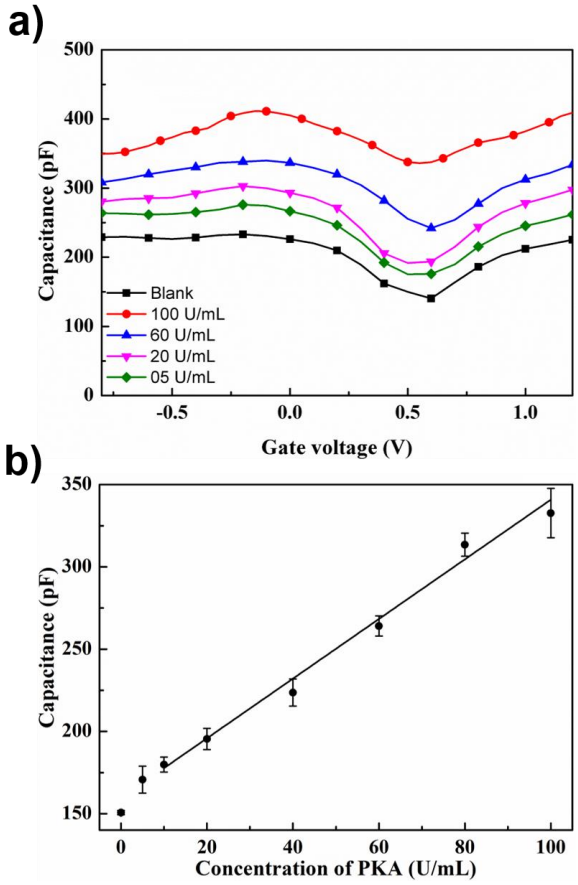

Fig. 4. (a) Set of C-V curves for the detection of PKA on aptamer modified MIS sensor. (b) The response of MIS sensor (n=3) at V_G=0.5 V for the detection of PKA. Buffer: 2 mM Tris-Cl saline, pH: 7

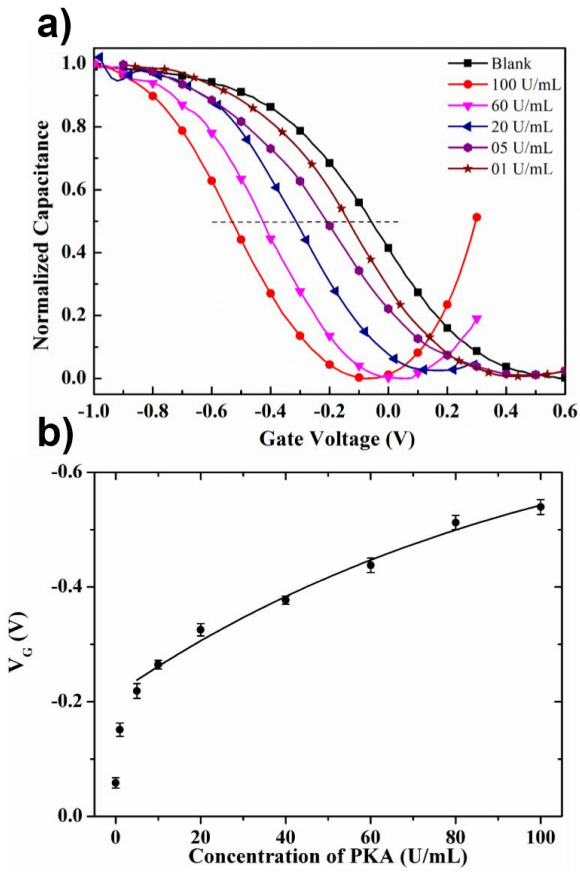

Fig. 5. (a) Set of C-V curves for the detection of PKA on aptamer modified EIS sensor. (b) The calibration curve of EIS sensor (n=3) for the detection of PKA. Buffer: 2 mM Tris-Cl saline, pH: 7

MPTS as the linker molecule. Fig. 3(c) shows the surface analysis of EIS sensor to confirm the attachment of AuNPs. The surface of the EIS sensor was characterized by the scanning electron microscopy. As evident from the image, highly resolved AuNPs with an average size of 16 ± 2 nm were attached on the SiO_2 surface. The AuNPs played a dual role in this EIS sensor. Along with enhancing the sensitivity by acting as additional gates, they also provided sites for immobilization of aptamer on their surface. The scheme for PKA analysis using EIS sensor is summarized in Fig. 2(b). In summary, the aptamers were immobilized on the monolayer of AuNPs. Next, different concentrations of PKA solution in 1X reaction buffer was dropped on the sensor surface for interaction with the aptamer.

B. Response of aptamer immobilization on sensor surface

The 20 amino acid long peptide is a well-documented aptamer for PKA. This aptamer inhibits the protein kinase competitively with a K_i = 2.3 nM [14, 17]. The isoelectric point of the aptamer is around pH 9.5, therefore it is positively charged at the neutral pH value [18]. Fig. 3(a) shows the capacitive response of MIS sensor after immobilization of positively charged aptamer. In this MIS sensor, the immobilization of aptamer produced no noticeable change in the flat-band voltage of the MIS capacitor. However, the immobilization of charged aptamer and PKA increased the surface area and density, thereby increasing the absolute capacitance of the MIS capacitor. The capacitance of the MIS sensor at V_G=0.5 V increased from 120 pF (bare Au) to 150 pF, after immobilizing the aptamer.

Fig. 3(b) shows the capacitive response of EIS sensor after immobilization of negatively charged citrate-capped AuNPs and after immobilization of positively charged aptamer. On the contrary of MIS sensor, the immobilization of charged molecules in this EIS sensor altered the flat-band voltage. The properties of EIS depend on the voltage applied to the gate insulator and charge on the surface of the gate insulator. The charge on the local surface of gate insulator affects the depletion region, thereby changing the flat-band voltage of the EIS capacitor. The attachment of negatively charged AuNPs to the SiO_2 surface shifted the gate voltage (V_G) towards more positive. A large shift in the V_G was obtained due to the charge of citrate-capped on AuNPs. Furthermore, the immobilization of positively charged aptamer neutralized the negative charge of the AuNPs to a certain level, thus shifting the V_G towards the negative direction.

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C. Capacitive detection of protein kinase A

The sequence of the aptamer is complementary to the binding site of the PKA. Therefore, aptamer acts as a pseudo-substrate for the enzyme. The pI of the PKA is pH 8.84, therefore it positively charged at the neutral pH. The interaction of aptamer with PKA changes the structure and total charge and charge distribution of the complex. This change in the charge and surface density on the sensor surface can be recorded using C-V analysis for label-free detection of PKA. Different concentration of PKA in 1X reaction buffer was allowed to interact with 100 µm aptamer modified surface for 20 minutes at 25 ℃. After the aptamer-PKA interaction, the reservoir was filled slowly with the tris buffer saline (TBS, 2 mM tris, 20 mM NaCl, pH 7) and then the C-V curves were analyzed. The strength of the buffer was kept low and the pH was set to 7 to reduce the effects of ions on the electrical response of the sensor.

Fig. 4(a) shows the C-V curves for label-free capacitive detection of PKA on MIS sensor. PKA solutions with a concentration range of 5 to 100 U/mL were detected using the MIS sensor. The capacitance of the MIS sensor increased gradually with the increasing concentration of the PKA. As discussed earlier, the immobilization of aptamer and then its interaction with the PKA increases the surface density, thus increasing the capacitance. The MIS sensor with immobilized aptamer produced a capacitance of 150 pF. Due to the interaction of aptamer with 100 U/mL of PKA, the capacitance increased to 335 pF. Fig. 4(b) shows the response of MIS sensor with respect to the different concentration of PKA. The error bar represents the standard deviation of three independent analysis. A linear and proportional increase in the capacitance was seen with the increasing concentration of PKA. Analysis of blank sample (only PKA reaction buffer) and non-specific targets (Fig. S1) produced no noticeable change in the signal, proving the specificity of the aptamer. The MIS sensor showed a limit-of-detection (LOD) of 5 U/mL (S/N=3) and a linear range from 10 to 100 U/mL ($R^2 = 0.99$).

Fig. 5(a) shows the C-V curves of the EIS sensor for different PKA concentration. A range of 1 to 100 U/mL of PKA was detected on the EIS sensor. As can be seen from Fig. 5(a), with the increasing concentration of PKA, the V_G gradually shifted more towards the negative. The local change in the charge of EIS sensor surface created a shift in the flat-band voltage. Detection of 100 U/mL of PKA shifted the V_G from -0.059 V to -0.54 V. Fig. 5(b) shows the relationship between the concentration of PKA and the obtained V_G. The EIS sensor showed a linear and proportional shift in the V_G with respect to the PKA concentration. The error bar represents the standard deviation of three independent analysis. Interaction of aptamer-PKA on EIS sensor produced a relative linear range from 5 to 80 U/mL ($R^2 = 0.98$), with a detection limit of 1 U/mL (S/N=3). The EIS sensor showed a lower detection limit in comparison with the MIS sensor. The high sensitivity of the EIS can be attributed to the presence of AuNPs and free SiO_2 surface. The textured EIS surface increased the surface area of the sensor for the immobilization of aptamers [19]. In addition, due to the absence of Au thin-film, any change in the surface charge directly affected the electronic property of the SiO_2-Si interface. Nevertheless, the MIS sensor and EIS sensor showed comparable and good sensing ability for the detection of PKA. Recently, an EIS sensor for protein kinase C based on protein phosphorylation assay was reported [20]. The sensor showed a detection limit of 10 U/mL, which is higher than the present report. Similarly, an ion-sensitive field-effect transistor sensor for creatine kinase II and carbon nanotube field-effect transistor sensor for PKA showed a detection limit of 0.0015 U/mL and 0.0012 U/mL, respectively [21, 22]. However, their detection depends on the kinase-catalyzed phosphorylation of peptide with very small detection range. The average concentration of extracellular PKA in humans is 2.15 U/mL. In cancer patients, the extracellular PKA concentration increases up to 20 U/mL [23] and the total PKA concentration is many-folds higher. The detection range of the proposed MIS or EIS sensing platforms lies within the physiological range. Therefore, either of them can be used or multiplexed with other sensors to achieve a PKA biosensor. Based on these results, we propose label-free and one-step capacitive sensing platforms for the detection of protein kinase A.

TABLE I
DETECTION OF PKA IN SPIKED CELL SAMPLE ON MIS SENSOR

Enzyme added (U/mL)	Capacitance expected (pF)	Capacitance obtained (pF)	Recovery (%)
0	150	178	117
60	255	266	104
100	337	342	101.5

D. Capacitive detection of protein kinase A in cell sample

To demonstrate the potential practicality of proposed sensor, the PKA detection was performed in the presence of human cell line. Conditioned medium containing LNCaP cell was used to mimic a biological specimen. The interaction of aptamer and PKA present in the spiked cell sample was analyzed using C-V curves. Table I summarizes the analyses, demonstrating good recoveries with respect to the concentrations of spiked PKA. A marginally elevated signal was obtained which is likely due to the extracellular PKA or interference of cell. In spite of that, the device showed high signal to noise ratio with MIS sensor precisely distinguishing between the spiked PKA and other interfering molecules. Therefore, these sensors can be used to detect PKA in cell samples or serum from patients.

IV. CONCLUSION

In conclusion, we fabricated capacitive sensing platform based on MIS and EIS sensor for the detection of PKA. One-step detection of PKA was achieved using aptamers immobilized on Au thin-film and AuNPs coated MIS sensor and EIS sensor, respectively. Interaction of aptamer and PKA on MIS sensor increased the absolute capacitance of the device and produced a detection limit of 5 U/mL for PKA. On the contrary, aptamer-PKA interaction on EIS sensor produced a shift in the gate voltage and showed a detection limit of 1 U/mL for PKA. The higher sensitivity of EIS sensor is attributed to the textured SiO_2 surface. Nevertheless, both the capacitive platform demonstrated an efficient sensing capability. The capacitance-based sensor enabled label-free and sensitive detection of PKA. Future work will be focused on the

development of a practical low-cost and integrated microchip with EIS sensor for the detection of PKA.

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