



Divalent ion-induced aggregation of gold nanoparticles for voltammetry Immunosensing: comparison of transducer signals in an assay for the squamous cell carcinoma antigen

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Abstract

A method is described for the electrochemical determination of squamous cell carcinoma (SCC) antigen, and by testing the effect of 30 nm gold nanoparticles (GNPs). Three comparative studies were performed in the presence and absence of GNPs, and with agglomerated GNPs. The divalent ion Ca(II) was used to induce a strong agglomeration of GNPs, as confirmed by colorimetry and voltammetry. Herein, colorimetry was used to test the best amount of salt needed to aggregate the GNPs. Despite, voltammetry was used to determine the status of biomolecules on the sensor. The topography of the surface of ZnO-coated interdigitated electrodes was analyzed by using 3D-nano profilometry, scanning electron microscopy, atomic force microscopy and high-power microscopy. The interaction between SCC antigen and antibody trigger vibrations on the sensor and cause dipole moment, which was measured using a picoammeter with a linear sweep from 0 to 2 V at 0.01 V step voltage. The sensitivity level was 10 fM by 3σ calculation for the dispersed GNP-conjugated antigen. This indicates a 100-fold enhancement compared to the condition without GNP conjugation. However, the sensitivity level for agglomerated GNPs conjugated antibody was not significant with 100 fM sensitivity. Specificity was tested for other proteins in serum, namely blood clotting factor IX, C-reactive protein, and serum albumin. The SCC antigen was quantified in spiked serum and gave recoveries that ranged between 80 and 90%.

Keywords Dielectric sensing · Zinc oxide · Interdigitated electrode · Colorimetry · Agglomeration · Dispersion · Blood disease · Cancer · Biosensor

Introduction

Squamous Cell Carcinoma (SCC) has been found to associate with various kinds of skin carcinoma, one of the world's most common diseases and the second largest form of skin carcinoma [1–3]. There are several types of SCC, which can be

categorized based on the anatomical location where cancer appears and often found in head, skin, cervix, esophagus, neck and lung and rarely noticed in pancreas, thyroid, prostate and bladder [4]. It was reported that SCC antigen (SCC-Ag) is the best biomarker for the identification of cervical cancer and has been shown that the existence of SCC-Ag is in the cervix epithelium with differential expressions, indirectly proves the occurrence of cervical cancer [5, 6]. Albeit, early treatment and prevention, better diagnosis, appropriate treatment and well-tracking of the disease progression, are much warranted.

The above situation makes the mandatory to reveal the detection of SCC-Ag along with other biomarkers in different sensing approaches [5–9]. Nanomaterial is the outstanding in the applications of nanotechnology, widely implemented in medical department to fulfill the demands. Among all types of nanomaterial, the mainly highlighted nanomaterial for the robust construction and efficient biosensor was gold nanoparticle (GNP) [10] due to its easier production and modification with biomolecules. GNPs combine high surface area and surface energy with a high conductivity and offers a large number

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of functional groups on the surface, enables particles to be easily functionalized [11–13]. In the fast-blooming protein detection area, metal nanoparticles [14] have huge approaches, because of their outstanding shape-dependent optical properties and the size is comprising good light scattering and absorption [15–17]. Despite, a study was reported on the determination of SCC using photoelectrochemical immunosensor. Several modifications were done to generate an ideal system for SCC determination. For example, integration of Molybdenum diselenide (MoSe_2) and hollow gold nanospheres (HGNs) nanocomposites are to modify the electrode surface for the better immobilization of SCC antibody. However, very least work was conducted using MoSe_2 and implementing such nanosheets is quite risky task [8].

Additionally, aggregation and dispersion of GNPs as a strategy to maximize the signal enhancement was utilized to conduct a comparative study for examining the state with a great detection. In colorimetric biosensors GNPs are frequently utilized for the effective detection [18]. According to Gopinath et al. [19], monovalent- and divalent-ions, oligo-strand and length and molecular conformations are the considered factors in colorimetric assays using GNPs. Dispersion and aggregation states of GNPs are depending on ionic salts, however, monovalent- (NaCl) and divalent-ions (CaCl_2 , MgCl_2 , NaCl), which reveal the similar effects on color changes. In the current study divalent ions CaCl_2 was used as the ion for the aggregation of GNPs, due to its relevance with the blood biomarkers. Generally, GNPs have negative surface charges and repulsion occurs among similarly charged GNPs, which avoid the force of attraction and causing no aggregation. Upon addition of salt, GNPs aggregate due to the presence of oppositely charged (Na^+ or Ca^{2+}) ions [20].

In the current study, SCC-Ag was engaged with antibody immobilized GNPs and functionalized on dielectrode sensor, operates based on interdigitated electrode (IDE). On IDE, comparative study was carried out using two different states of GNPs. Aggregated and dispersed GNPs were conjugated with anti-SCC antibody to monitor the differences occur in electrical measurements and validated the current changes due to the antigen-antibody interaction on assembled and dispersed GNPs. The primary aim of this research was to develop a device for determining the abundance SCC at a lower limit of detection. It may assist in early diagnosis [19] and is expected to decline the mortality rate due to SCC.

Material and methods

Materials and reagents

SCC-Ag was procured from RANDOX Life Sciences, Malaysia (<https://www.randox.com/tag/malaysia/>). Anti-SCC

antibody was received from Next Gene (Malaysia). (3-aminopropyl)triethoxysilane (APTES) and GNPs were purchased from Sigma Aldrich, USA (<http://www.sigmaaldrich.com/united-states>). N-Hydroxysuccinimide (NHS), 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and 16-mercaptohexadecanoic acid (16-MDA) were from GE Healthcare (https://be.vwr.com/assetsvc/asset/fr_BE/id/17891730/contents). Phosphate Buffer Saline (PBS) was obtained from Sigma Aldrich, USA (<http://www.sigmaaldrich.com/united-states>) for washing and binding purposes, while ethanolamine was from Fisher Scientific, UK (<https://www.fishersci.com>) as the blocking agent. Calcium chloride (CaCl_2) was received from Sigma Aldrich, USA (<http://www.sigmaaldrich.com/united-states>) for salt to aggregate GNPs. The following parameters were optimized: (a) CaCl_2 concentration; (b) GNPs; (c) SCC-Ag concentration. Respective data and figures are given in the [Electronic Supporting Material](#).

Interdigitated electrode (IDE) fabrication

IDE is a comb structure manufactured with micro-dimension terminal arrays, which can be categorized under the electrochemical sensor. IDE is cheap and facile to be utilized in a wide range of applications due to easier and flexible production method. The fabrication procedure of IDE includes cleaning, oxidation and traditional photolithography processes. Initially, p-type silicon wafer was rinsed by piranha solution (mixture of sulfuric acid and hydrogen peroxide in 3:1 ratio) then rinsed with distilled water. Wet oxidation process was carried out to create an oxide layer on the surface of silicon wafer. Later, aluminum deposition was done using a thermal evaporator. The photoresist coating procedure was performed by the spin-coating. Next, the patterning process on aluminum decorated IDE was performed using the first mask by UV-exposure for 10 s. The etching process was carried out to remove the aluminum on unwanted area followed by ZnO thin film deposition was performed using the sol-gel method. The sensor had undergone annealing process in furnace to transform the seed solution into nanoparticles. Later, the photolithography process was continued by exposing the sensor to the second mask. Finally, the sensor was monitored under High-power Microscope (HPM) to make sure the design of IDE is well prepared.

Sensing surface functionalization

To remove the foreign particles on the active surface, IDE was initially rinsed by 70% alcohol. Then, 2% APTES was loaded on the active surface of IDE to generate the amine-group [21] and incubated at ambient temperature for 1 h. Using 10 mM of PBS (pH 7.4) [22], the unbound APTES on the surface was washed away.

Surface characterization analysis

Surface topography analysis was performed using Scanning Electron Microscopy (SEM; JSM-6010LV) under a high-energy electron beam. The beam was scanned over the surface and the scattered electrons were observed. The sample used was conductive electrically and under the vacuum. Atomic Force Microscopy (AFM: SPA400-SPI4000) from Seiko Instruments Inc., Japan was used to inspect the dielectric surface morphology after the attachment of GNPs. HPM (Olympus BX51) with the optional resolutions of 5x, 20x, 150x, 250x, (Olympus, Japan) were utilized to provide the further supporting analysis, which reveals a clear cut image on electrodes. In addition, a 3D nano-profilometry (Hawk 3D Optical Surface Profiler) was obtained from Pemtron Co., Ltd., South Korea and utilized to observe the sharp edges of the electrodes as well as the bandgaps between the electrodes. Respective data and figures are given in Electronic Supporting Material (Fig. S1).

Enzyme-linked Immunosorbent assay (ELISA)

Initially, ELISA 96-well polystyrene plate was coated with different concentrations of SCC Ag (10 fM, 100 fM, 1 pM, 10 pM, 100 pM, 1 nM, 10 nM, 100 nM), which were mixed with 1x of coating buffer and incubated for overnight at 4 °C. As a negative and specificity controls, wells were also coated with coating buffer, C-reactive protein and Factor IX antigens. Later, low salt washing buffer (Tris Buffer saline Tween; TBST) was utilized to remove the unbound antigen followed by blocking the well with 2% of BSA for 1 h was done. The primary anti-SCC with 1:1000 dilution was interacted into each well and incubated for 30 min followed by the secondary antibody-conjugated HRP at 1:1000 dilution was reacted into each well. Washing steps were consecutively carried out between each step using TBST. At the end, substrate (TMB One Solution) was dropped into each well to monitor the color changes were occurred due to antigen-antibody interaction. The absorbance was measured at 450 nm using UV-Vis spectrophotometer. Photograph was taken 10 min after the addition of the substrate.

Analytical high-performance

To evaluate the selective detection, anti-SCC modified surface was interacted with (Factor IX, C-reactive protein and serum albumin) independently, which can be found abundantly in the human serum. After the incubation, the current changes were noted and the washing steps were carried out as described above. Similarly, humans serum with 1:1000 dilution was injected on the anti-SCC modified surface to carry out the specificity analysis. In addition, 10 and 100 fM of SCC-Ag were spiked into the human serum independently and

evaluated on the surface. Precise procedure to perform with real sample is provided in Electronic Supporting Material.

Results and discussion

Three different comparative studies were carried out to investigate the better and accurate detection system for SCC. First study was done by modifying the active surface with anti-SCC and interacted the SCC-Ag. In the second case, gold was conjugated with anti-SCC-Ag before immobilization on IDE surface; while in third comparative study was performed using the aggregated GNPs to enhance the sensing procedure. These three studies were carried out in ambient temperature under the wet condition to maintain the activity of the biological molecules. The optimization experiments were carried out and found the ideal conditions with: (a) CaCl_2 concentration; (b) GNPs amount; (c) antibody concentration. Respective data and figures are given in the Electronic Supporting Material (Fig. S2). In current study, linear voltammetry technique was used to justify that this technique was also another eye catching technique for researchers to conduct the detection with high selectivity and sensitivity. Linear voltammetry is measuring the current response at the fixed potential. Numerous studies were published using a linear voltammetry technique, which reveals the outstanding results [23–25]. For example, C-reactive protein detection in human serum was successfully carried out using a linear voltammetry [26]. Thus, the obtained reading was utilized to calculate the limit of detection more precisely. Albeit, another study was utilized the similar technique to monitor the accuracy of the fabricated device and the consistency in current flow [27].

Choice of material

Notably, transducer is the part of biosensor which transforms the biological signal into the quantifiable electrical signal. ZnO as the elite nanomaterial was utilized in the current study for the novel sensor development. ZnO is categorized as semiconducting matrix, which displays a high surface to volume ratio. Further, it has broad energy band gap with 3.37 eV followed by 60 meV of bond energy at ambient temperature [28]. ZnO is nontoxicity nanomaterial with a high chemical stability. Implementation of ZnO as transducer eases the electron transfer without the need of intermediates with a high electron mobility. Though, GNP was conjugated with antibody in this work to capture more antigens for the better sensitivity. GNPs can ease the electron movement in a single way direction on the sensor surface. Generally, protein adsorption on the electrodes surface may leads to the drastic structural changes and decline the biological activity [29]. However, integration of GNPs can assist to retain the properties of biomolecules due to their high biocompatibility and the surface

free energy. GNPs combine high surface area and surface energy with a high-conductivity and offer a large number of functional groups on the surface, enabling particles to be easily functionalized [25].

Surface topography observation

Topography analyses of agglomerated against dispersed GNPs were performed using HPM, SEM and AFM. Figure 1a&b displays the observed images by HPM. It was supported by SEM analysis (Fig. 1c&1d) and it apparently displays the image of dispersed and aggregated GNPs, respectively. Further, GNPs imaging was validated by AFM analysis and there were apparent evidences for dispersion and aggregation of GNPs. Figure 1e represents the dispersed state of GNPs, while Fig. 1f represents the aggregated state of GNPs.

Enzyme-linked Immunosorbent assay (ELISA)

ELISA was executed to countersign the detection of SCC using a biosensor at a lower detection limit, low amount of sample required, fast and accurate results obtained. ELISA known to be a standard method for the protein identification and protein-protein interactions by utilizing appropriate antibodies [30]. This technique was performed to study the genuine interaction between

SCC-Ag and anti-SCC-Ag and to attest the specificity by immobilizing non-specific protein (CRP and Factor IX). Initially, the wells were coated with different concentrations of antigen, which was diluted using coating buffer. Herein, coating buffer was utilized instead of PBS in order to promote the better adsorption on the ELISA surfaces and maximize the antigen-antibody binding event. BSA was coated to avoid non-specific binding and able to stabilize the surface-bound biomolecules on the well [31]. Later, anti-SCC was added to form antigen-antibody complex followed by antibody-conjugated enzyme (HRP) to create the signal when enzyme-substrate reaction. Hence, color changes occur due to the enzymatic reaction, which indicates the interaction had occurred between antigen and antibody. UV-Vis measurements were done at 405 nm to measure the absorbance changes among different concentrations of SCC-Ag. Apparent color change was observed at 100 nM, while mild change in color was observed in 1 nM (Fig. 2a). Despite, there is no prominent change with color in control and with other concentrations. The absorbance results clearly show that 100 nM has a great absorbance and the clear binding begins at 100 nM. These results show that the sensitivity range falls between 1 and 100 nM. Therefore, using this sensitivity level the experiment was further continued for SCC-Ag and anti-SCC-Ag interaction by the electrical measurements.

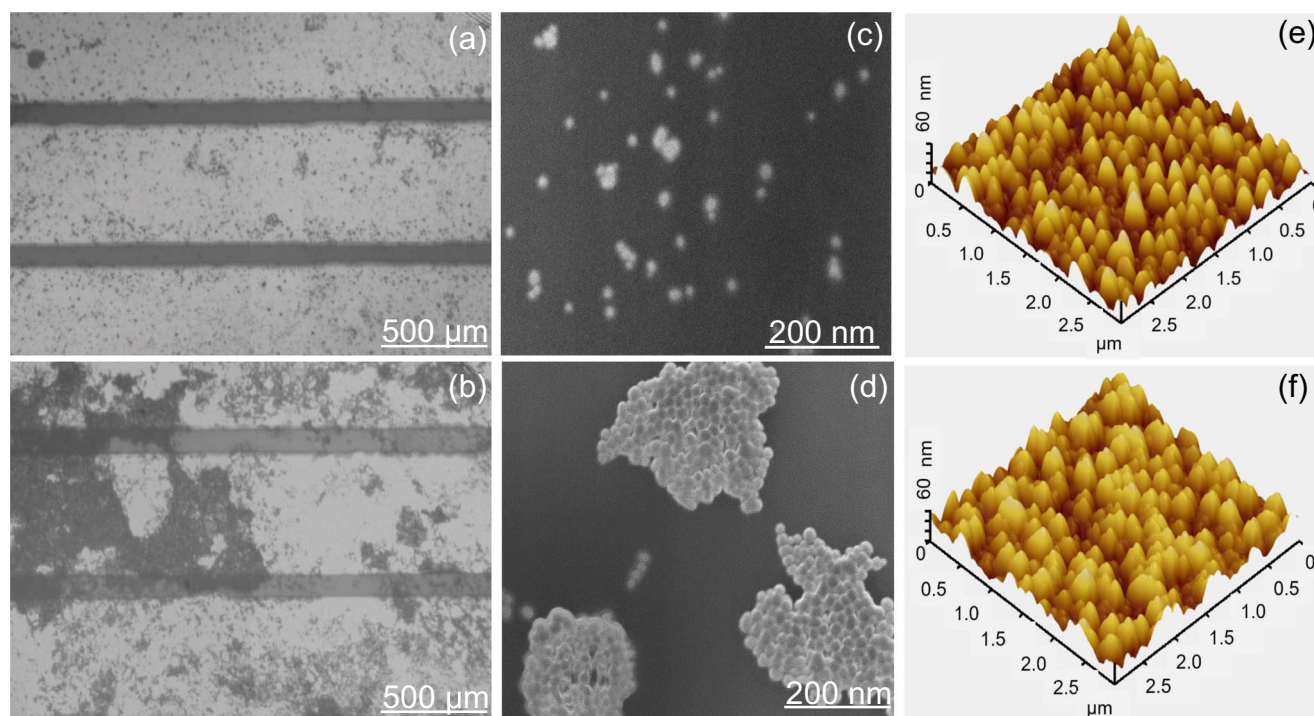


Fig. 1 Morphological inspection for dispersed and agglomerated GNPs on the active surface area of the sensor. 3D Surface Nano-profilometry observation with (a) dispersion and (b) agglomeration; (c) Scanning

Electron Microscopy observation with dispersion and (d) agglomeration; Atomic Force Microscopy observation with (e) dispersion and (f) agglomeration

Interactive study by voltammetry between anti-SCC-Ag and SCC-Ag

Initially, surface modification was carried out by injecting APTES on the surface of IDE. APTES has been widely utilized in biosensing platform due to its bifunctional nature. APTES serves as a superglue to anchor biomolecules on the solid surfaces and allows binding of more biomolecules during the biomolecular assembly with a higher specificity [32]. The need of APTES is higher in biosensing platforms because of its outstanding characteristics, such as able to maintain biomolecular stability, reduced biofouling and the enhanced analytic performance. Herein, silanized platform with APTES was created.

Voltammetry measurement was started with a bare device and it gives the current reading about 5.08×10^{-5} A. Then, APTES was injected and shows decrement in reading to 1.67×10^{-5} A. Then, anti-SCC-Ag was immobilized and the current flow was noted as 8.72×10^{-6} A, lower than the bare device (Fig. 2b). These current changes were due to the charge variations among the molecular assembly. In addition, the stability analysis in Fig. 2b (inset) shows that the sensor used in this work has the similar capacity as the current high-performance biosensors in the medical applications (Table 1). The interactive study has related data and figures are given in the Electronic Supporting Material (Fig. S3a).

Interactive study by voltammetry between SCC-Ag and GNPs conjugated anti-SCC-Ag

This study was conducted to enhance the sensing mechanism using GNPs, because they have capability to capture more antibodies for antigen interaction. As stated above 30 nm

GNPs were used in this study to increase the surface-to-volume ratio. GNPs have been extensively utilized because their surface is enabling different molecules to be easily functionalized or covalently attaches to the sensing surface. Normally, proteins adsorption on the electrode surface leads to the drastic structural changes and decline the biological activity. Integration of GNPs can assist to retain the properties of biomolecules, due to their high-biocompatibility and the surface free energy characteristics [17]. Initially, ZnO was coated on the electrode surface followed by APTES. ZnO surface can be chemically functionalized by APTES by the high electrostatic and hydrophobic attractions. Herein, APTES was bonded with hydroxyl group on ZnO modified sensor surface, which generates amine-functionalized layer by linking the hydroxyl group. Then, thiolated GNPs [16] with carboxyl groups at the end was quenched with EDC and NHS to activate the carboxyl group for antibody binding. This mixture was dropped on the sensor surface to create bond between the amine-functionalized surface and GNPs. Then, the specific antigen capturing event was carried out. Respective data and figures are given in the Electronic Supporting Material (Fig. S3b).

Evaluation of interaction between SCC-Ag and CaCl_2 -induced assembly of GNPs conjugated anti-SCC-Ag by voltammetry

Conditions were followed as in the above cases and all the steps were conducted in wet condition because biomolecules are inactive in dry condition and liquid medium is needed in biosensing platform for the electron movement in order to obtain the accurate current reading upon antigen interaction. Respective data and figures are given in the Electronic Supporting Material (Fig. S3c).

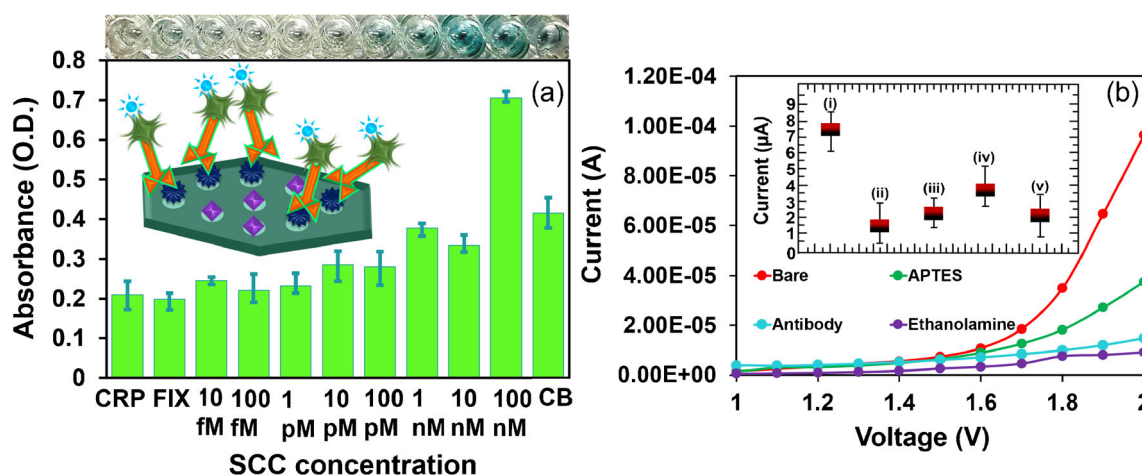


Fig. 2 **a** Enzyme-linked-Immunosorbent Assay. The color development with the substrate is shown by a photograph. Diagrammatic representation is shown by the figure inset. The bar graph represents the absorbance value for each concentration. **b** Voltammetry current analysis

prior to surface modifications. Figure inset displays the performance of the sensor; (i) Bare device; (ii) APTES; (iii) EDC and NHS; (iv) Anti-SCC antibody; (v) Ethanolamine. Error bars indicate the averaged values by the replicates ($n = 3$)

Analytical high-performance analysis

A linear regression graph (concentration versus current) was plotted for all three comparative studies. First, investigation without the implementation of GNPs shows increment in current from the lowest to the highest concentrations, which means from femto- to the nano-molar ranges, and the current response was almost approaching 1 pM of detection limit using 3σ calculation (Fig. 3a) is in the range of ± 0.5 to 2.5×10^{-5} A. Though the detection limit for gold-conjugated anti-SCC-Ag is almost proceeding towards 10 fM using 3σ calculation (Fig. 3b) is in the range of ± 1.0 to 3.0×10^{-5} . Whereby, $1.45 \mu\text{A}\cdot\text{M}^{-1}\cdot\text{cm}^{-2}$ sensitivity was achieved with 1.38 cm^2 of electrode active surface area. Here, Randle-Sevcik equation was utilized to calculate the electrode active surface area. The current dependence square root of scan rate was plotted (Fig. S4) to identify the slope, which represents the diffusion coefficient value [34] is needed for Randle-Sevcik equation. Respective data is given in the Electronic Supporting Material. In addition, it shows 100 fold drastic enhancement when compared between existence and non-existence of GNPs. Herein, the linearity graph (Fig. 3c) demonstrates the detection limit is almost proceeding to 100 fM, in the range of ± 0.2 to 2.0×10^{-5} A for the detection by utilizing agglomerated GNP, which is higher than the previous system. Thus, it proves that disperse state GNPs have a greater capability in antigen capturing compared to aggregate GNPs.

The performance of sensor was tested and presented in Fig. 3d. The specificity of the sensor was monitored using different serum proteins, such as C-reactive protein (CRP), human blood clotting factor IX (FIX) and human serum albumin (HSA). Herein, the reference material would be the commercially purchased antibody because only specific antigen will bind to the antibody and cause the current to increase drastically due to the dipole moment created by antigen-antibody binding. When the current increases above the antibody attached level means there is dipole moment is due to the

specific antigen-antibody binding. Despite, non-specific protein like Factor IX, C-reactive protein and serum albumin were dropped independently to test the specificity and there are slight increments, which show non-specificity and not significant. Real and spiked sample analysis showing the drastic increment is due to abundance of SCC antigen in human sera. Initially, CRP was interacted on the anti-SCC-Ag antibody modified sensor and the current flow was recorded. Later, Factor IX and HSA were dropped on the IDE surface and the current flow was monitored independently. The concentration of CRP in healthy human was in the range of below $3.0 \text{ mg}\cdot\text{L}^{-1}$ [27], while Factor IX does exist in human serum about $3\text{--}5 \text{ mg}\cdot\text{L}^{-1}$ [15]. While, in healthy human serum HSA is about $45 \text{ mg}\cdot\text{mL}^{-1}$ [5]. In this study, HSA reveals $\sim 2.02 \times 10^{-7}$ A and FIX gives reading $\sim 2.07 \times 10^{-7}$ A, while CRP gives $\sim 2.15 \times 10^{-7}$ A. Inspecting the current reading, these three proteins reveals a least current difference from the antibody. The analytical system clearly shows the efficient SCC-Ag detection by anti-SCC-Ag antibody compared to other proteins and discriminates.

Despite, SCC-Ag does contain in human sera at the range of $\leq 1.5 \text{ ng}\cdot\text{mL}^{-1}$ [5]. Hence, in the current study the abundance of SCC-Ag in human serum was tested. The human serum with 1:1000 dilution has been dropped on the antibody surface and the current reading shows decrement from the level of human serum spiked with 10 fM and 100 fM of SCC-Ag. This is due to the low concentration of SCC-Ag available in the human serum. SCC-Ag-spiked human serum at 10 fM and 100 fM were injected independently on the antibody surface and the current readings found were 3.07×10^{-7} A and 3.44×10^{-7} A, respectively. This increment was due to the abundance of SCC-Ag in the human serum (Fig. 3d). There are other related proteins for the determination of SCC. For example, immunosuppressive acidic protein (IAP) highly associates with cervical cancer. A study was conducted for the measurement of IAP serum together with SCC determination. This study concluded that the increasing level of IAP leads to the elevation of SCC. Herein, the sensitivity level

Table 1 Comparison among the currently available SCC detections by different sensing systems

Method Applied	Material	Times of analysis	Detection limit ($\text{pg}\cdot\text{mL}^{-1}$)	Reference
Photoelectrochemical immunosensor	MoSe ₂ nanosheets	n = 3	0.21	[8]
Photoelectrochemical immunosensor	BiOBr/Bi ₂ S ₃ heterostructures	n = 3	0.3	[9]
Photoelectrochemical immunosensor	Carbon intercalated MoS ₂ hollow nanospheres (C/MoS ₂)	—	1.8	[6]
Fluorescence-based immunosensor	3D Carbon nanotube	—	500	[33]
Amperometry	Strontium oxide	—	450	[5]
Electrochemical	Pillar[5]arene-Pd/MoS ₂ nanocomposites	—	0.14	[7]
Amperometry	Zinc Oxide	n = 3	0.45	Current work
ELISA	—	n = 3	4,500,000	Current work

reaches up to 87.3% for both IAP and SCC. Another study was conducted to monitor the expression of highly sensitive C-reactive protein and CA-125, whereby both proteins are related to cervical cancer recurrence. Here, the recurrence sensitivities were 0.65 and 0.74 for both C-reactive protein and CA-125, respectively [35].

Based the current work several limitations were encountered. Like, 30 nm GNP was utilized in this assay for the better detection. Literature says that smaller sized GNPs with 10–15 nm are more stable compared to the larger one [19]. Additionally, only need a lower amount of antibody to coat the smaller sized GNPs. Thus, low concentration of antigen needed to bind with antibody hence can lower the detection limit. Secondly, GNPs are relatively expensive material which may cause financial issue to conduct the research.

Henceforth, novelty and benefits of the current work was narrated clearly. Previously, NaCl was used widely in all types of applications with GNP aggregation. Herein, CaCl_2 was utilized for GNP aggregation, which is a divalent ion. In fact, CaCl_2 is optional candidate has optimum capability for GNP aggregation. Studies says that the effect of mono- and divalent ions are similar in the case of GNP aggregation [16].

Additionally, CaCl_2 have performed well in serum containing samples. Hence, this was utilized in the current system to enhance the sensitivity level of detection system. Secondly, colorimetric technique was utilized to study the capability of GNP in two different states: dispersion and aggregation in order to offer more surface-to-volume ratio for more molecular capturing to enhance the sensitivity level. Results were obtained clearly displaying that dispersed state of GNP holds a high capability for more antibodies capturing and able to lower the detection limit. Thirdly, electrical characterization was implemented to determine the best needed CaCl_2 concentration for the detection using aggregated GNP. The current changes were observed at 125 mM of CaCl_2 due to GNP aggregation hence continued with the detection procedure. Concerning the benefits, integration of GNP in biomolecules detection system can reveal good and quick outcome. Further, utilization of biosensor platform can obviously cut-off the medical cost and gives the outstanding results. A study was conducted recently for SCC-Ag detection by modifying the electrode with strontium oxide. The detection limit obtained was 10 pM [24]. Herein, the current study obtained 10 fM as the detection limit, which is 1000 folds lower than the previous study.

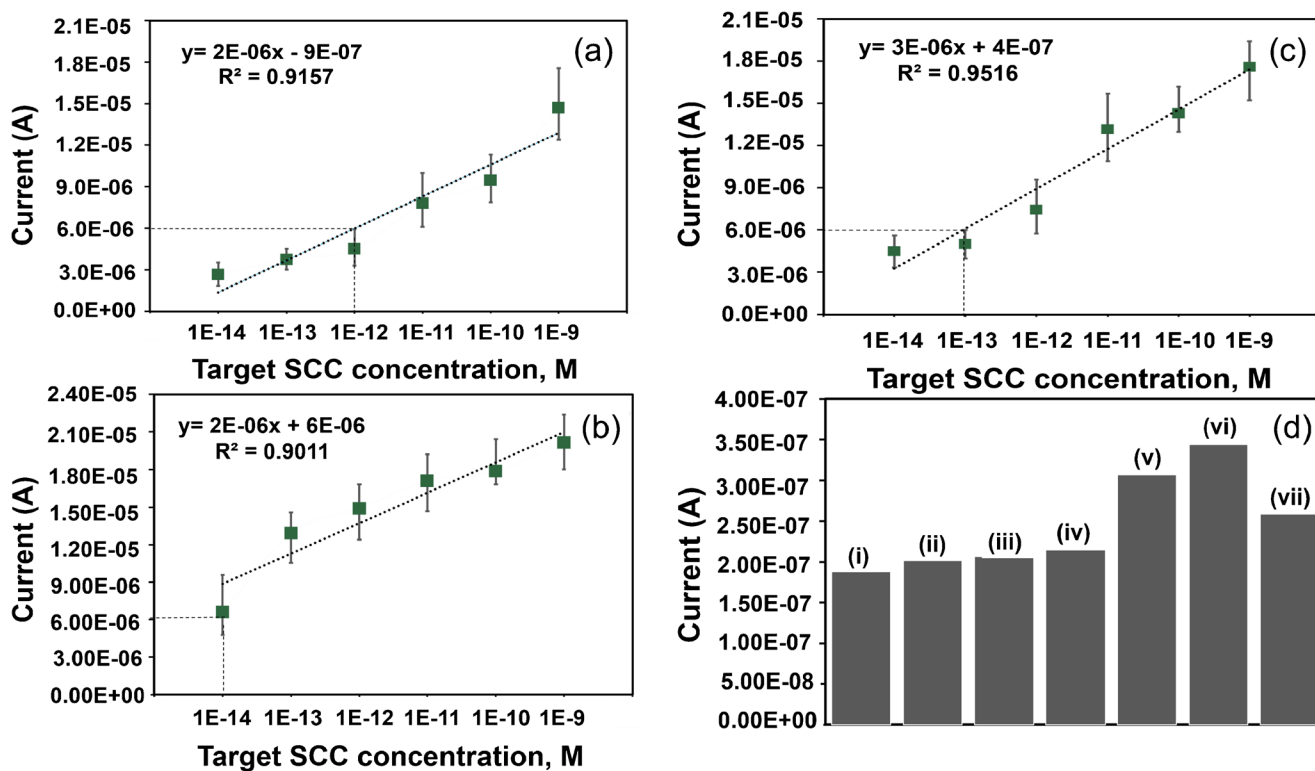


Fig. 3 Analytical performance analysis with; (a) Linear regression plot with averaged values from triplicates ($n = 3$) for the interaction of SCC-Ag and anti-SCC-Ag with the standard deviations are ranging from ± 0.5 to 2.5×10^{-5} A; (b) Linear regression plot with averaged values from triplicates ($n = 3$) for the interaction of anti-SCC-Ag-GNP complex and SCC-Ag with the standard deviations are ranging from ± 1.0 to 3.0×10^{-5} A; (c) Linear regression plot with averaged values from triplicates ($n = 3$)

for the interaction of anti-SCC-Ag-GNP (agglomerated) complex and SCC-Ag with the standard deviations are ranging from ± 0.2 to 2.0×10^{-5} A; (d) Specificity analysis; (i) Immobilized antibody; (ii) Serum albumin; (iii) Factor IX; (iv) C-reactive protein; (v) Human serum spiked 10 fM of SCC-Ag; (vi) Human serum spiked 100 fM of SCC-Ag; (vii) Human serum. The current to voltage (I-V) measurements were performed from 0 to 2 V

Conclusion

SCC is considered as a fatal causing disease and mostly affects women's health, thus an appropriate biomarker is much needed for high-sensing performance system. Herein, SCC-Ag was utilized as biomarker to detect and prevent the disease at the early stages in order to decline the care expenses for the sophisticated stage-related patient. Furthermore, a precise probe anti-SCC-Ag antibody was used and highlighted in this study to develop an accurate sensing mechanism on zinc oxide coated IDE sensor. To develop a high-performance sensing biosensor GNPs were integrated. GNPs hold a wide variety of key features, such as easier to modify on the sensor surface, biocompatibility and water dispersal. GNPs have the capability to improve the sensitivity and specificity, so that it can be considered as the foremost surface improvement. Though, the detection system using GNPs touches femtomolar range with a greatest specificity by discriminating other non-specific proteins and human serum. However, this detection system is not possible to perform if the voltammetry measurement is not accurate with the set-up. Less accuracy in voltammetry reading will be resulting in a wrong conclusion at the end. Despite, optimistic key features of this sensor can be performed without prior knowledge and lower amount of sample needed for a simple bedridden analysis. Biosensor with a precise probe will be the better platform for early detection of SCC-Ag which is mainly associated with cervical cancer.

References

- Kabir S, Schmults CD, Ruiz ES (2018) A review of cutaneous squamous cell carcinoma epidemiology, diagnosis, and management. *Int J Cancer Manag* 11:1–9. <https://doi.org/10.5812/ijcm.60846>
- Qin H De, Liao XY, Chen Y Bin, et al (2016) Genomic characterization of esophageal squamous cell carcinoma reveals critical genes underlying tumorigenesis and poor prognosis. *Am J Hum Genet* 98:709–727. <https://doi.org/10.1016/j.ajhg.2016.02.021>
- Voiculescu V, Calenic B, Ghita M et al (2016) From Normal skin to squamous cell carcinoma: a quest for novel biomarkers. *Dis Markers* 2016:1–14. <https://doi.org/10.1155/2016/4517492>
- Abnet CC, Arnold M, Wei WQ (2018) Epidemiology of esophageal squamous cell carcinoma. *Gastroenterology* 154:360–373. <https://doi.org/10.1053/j.gastro.2017.08.023>
- Wang H, Lakshmi priya T, Chen Y, Gopinath SCB (2019) Squamous cell carcinoma biomarker sensing on a strontium oxide-modified Interdigitated electrode surface for the diagnosis of cervical Cancer. *Biomed Res Int* 2019:1–7. <https://doi.org/10.1155/2019/2807123>
- Wang C, Wu T, Wang X et al (2019) Ultrathin-layered carbon intercalated MoS₂ hollow nanospheres integrated with gold nanoparticles for photoelectrochemical immunosensing of squamous cell carcinoma antigen. *Sensors actuators B Chem*:126716. <https://doi.org/10.1016/j.snb.2019.126716>
- Qian X, Zhou X, Qu Q et al (2019) Ultrasensitive and robust electrochemical sensing platform for the detection of squamous cell carcinoma antigen using water-soluble pillar [5]arene-Pd/MoS₂ nanocomposites. *Electrochim Acta* 313:235–244. <https://doi.org/10.1016/j.electacta.2019.05.032>
- Ye X, Li M, Hu Y et al (2018) Sensitive photoelectrochemical immunosensor for squamous cell carcinoma antigen based on MoSe₂ nanosheets and hollow gold nanospheres. *Sensors Actuators B Chem* 275:199–205. <https://doi.org/10.1016/j.snb.2018.08.010>
- Fan D, Bao C, Liu X, Feng J, Wu D, Ma H, Wang H, Wei Q, du B (2019) Facile fabrication of visible light photoelectrochemical immunosensor for SCCA detection based on BiOBr/bi 2 S 3 heterostructures via self-sacrificial synthesis method. *Talanta* 198: 417–423. <https://doi.org/10.1016/j.talanta.2019.02.043>
- Gopinath SCB, Lakshmi priya T, Md Arshad MK, et al (2019) Nanoelectronics in biosensing applications. Elsevier Inc.
- Pundir CS, Narwal V (2018) Biosensing methods for determination of triglycerides: a review. *Biosens Bioelectron* 100:214–227. <https://doi.org/10.1016/j.bios.2017.09.008>
- Nikolelis DP, Nikoleli GP (2018) Nanotechnology and biosensors. *Nanotechnol Biosens* 22:1–446. <https://doi.org/10.1016/C2017-0-00358-0>
- Lakshmi priya T, Fujimaki M, Gopinath SCB, et al (2013) PAPER a high-performance waveguide-mode biosensor for detection of factor IX using PEG-based blocking agents to suppress non-specific binding and improve sensitivity †. 2863–2870. doi: 10.1039/c3an00298e
- Kumar A, Boruah BM, Liang X (2011) Gold Nanoparticles: Promising Nanomaterials for the Diagnosis of Cancer and HIV / AIDS:2011. <https://doi.org/10.1155/2011/202187>
- Letchumanan I, Gopinath SCB, Arshad MKM, et al (2019) Gold Nano-urchin Integrated Label-free Amperometric Aptasensing Human Blood Clotting Factor IX: A Prognosticative Approach for “Royal Disease.” *Biosens Bioelectron* <https://doi.org/S095656631930106X>
- Gopinath SCB, Perumal V, Rao BS, Md Arshad MK, Voon CH, Lakshmi priya T, Haarindrapasad R, Vijayakumar T, Chen Y, Hashim U (2017) Voltammetric immunoassay for the human blood clotting factor IX by using nanogapped dielectrode junctions modified with gold nanoparticle-conjugated antibody. *Microchim Acta* 184:3739–3745. <https://doi.org/10.1007/s00604-017-2389-7>
- Lakshmi priya T, Horiguchi Y, Nagasaki Y (2014) Co-immobilized poly(ethylene glycol)-block-polyamines promote sensitivity and restrict biofouling on gold sensor surface for detecting factor IX in human plasma. *Analyst* 139:3977–3985. <https://doi.org/10.1039/c4an00168k>
- Anniebell S, Gopinath SCB (2018) Polymer conjugated gold nanoparticles in biomedical applications. *Curr Med Chem* 25:1433–1445. <https://doi.org/10.2174/0929867324666170116123633>
- Gopinath SCB, Lakshmi priya T, Awazu K (2014) Colorimetric detection of controlled assembly and disassembly of aptamers on unmodified gold nanoparticles. *Biosens Bioelectron* 51:115–123. <https://doi.org/10.1016/j.bios.2013.07.037>
- Fatin MF, Rahim Ruslinda A, Gopinath SCB et al (2019) Co-ordinated split aptamer assembly and disassembly on gold nanoparticle for functional detection of HIV-1 tat. *Process Biochem* 79: 32–39. <https://doi.org/10.1016/j.procbio.2018.12.016>
- Cheen OC, Gopinath SCB, Perumal V, Arshad MKM, Lakshmi priya T, Chen Y, Haarindrapasad R, Rao BS, Hashim U, Pandian K (2017) Aptamer-based impedimetric determination of the human blood clotting factor IX in serum using an interdigitated electrode modified with a ZnO nanolayer. *Microchim Acta* 184:117–125. <https://doi.org/10.1007/s00604-016-2001-6>
- Gopinath SCB, Perumal V, Kumaresan R, Lakshmi priya T, Rajintraprasad H, Rao BS, Arshad MKM, Chen Y, Kotani N,

- Hashim U (2016) Nanogapped impedimetric immunosensor for the detection of 16Å kDa heat shock protein against mycobacterium tuberculosis. *Microchim Acta* 183:2697–2703. <https://doi.org/10.1007/s00604-016-1911-7>
23. Letchumanan I, Gopinath SCB, Md Arshad MK, Anbu P, Lakshmi priya T (2019) Gold nano-urchin integrated label-free amperometric aptasensing human blood clotting factor IX: A prognostic approach for “Royal disease.”. *Biosens Bioelectron* 131: 128–135. <https://doi.org/10.1016/j.bios.2019.02.006>
 24. Lakshmi priya T, Gopinath SCB (2019) An introduction to biosensors and biomolecules. Elsevier Inc.
 25. Perumal V, Hashim U, Gopinath SCB, Haarindrapasad R, Foo KL, Balakrishnan SR, Poopalan P (2015) “Spotted Nanoflowers”: gold-seeded zinc oxide nanohybrid for selective bio-capture. *Sci Rep* 5: 1–12. <https://doi.org/10.1038/srep12231>
 26. Sin LL, Arshad MKM, Fathil MFM et al (2016) Zinc oxide interdigitated electrode for biosensor application. *AIP Conf Proc* 1733: 1–5. <https://doi.org/10.1063/1.4948893>
 27. Letchumanan I, Md Arshad MK, Balakrishnan SR, Gopinath SCB (2019) Gold-nanorod enhances dielectric voltammetry detection of c-reactive protein: a predictive strategy for cardiac failure. *Biosens Bioelectron* 130:40–47. <https://doi.org/10.1016/j.bios.2019.01.042>
 28. Cao L, Kiely J, Piano M, Luxton R (2018) Facile and inexpensive fabrication of zinc oxide based bio-surfaces for C-reactive protein detection. *Sci Rep* 8:12687. <https://doi.org/10.1038/s41598-018-30793-z>
 29. Adam H, Gopinath SCB, Md Arshad MK, Ramanathan S, Ashokkumar T, Azan MIA, Adam T, Hashim U (2019) Fabrication of gold nanorod–zinc oxide nanocomposite on gap-fingered integrated interdigitated aluminum electrodes and their response to electrolytes. *Appl Phys A Mater Sci Process* 125:1–11. <https://doi.org/10.1007/s00339-019-3106-7>
 30. Lakshmi priya T, Gopinath SCB, Hashim U, Tang TH (2016) Signal enhancement in ELISA: biotin-streptavidin technology against gold nanoparticles. *J Taibah Univ Med Sci* 11:432–438. <https://doi.org/10.1016/j.jtumed.2016.05.010>
 31. Xiao Y (2013) Enzyme-Linked. *J Immunol Methods* 384:148–151. <https://doi.org/10.1016/j.jim.2012.06.009.Enzyme-Linked>
 32. Victor dos Santos Junior C, Sader MS, Gonçalves GC et al (2018) Effect of pH on the adsorption and interactions of bovine serum albumin with functionalized silicon nitride surface. *Colloids Surfaces B Biointerfaces* 167:441–447. <https://doi.org/10.1016/j.colsurfb.2018.03.045>
 33. Song CK, Oh E, Kang MS, Shin BS, Han SY, Jung M, Lee ES, Yoon SY, Sung MM, Ng WB, Cho NJ, Lee H (2018) Fluorescence-based immunosensor using three-dimensional CNT network structure for sensitive and reproducible detection of oral squamous cell carcinoma biomarker. *Anal Chim Acta* 1027:101–108. <https://doi.org/10.1016/j.aca.2018.04.025>
 34. Shetti NP, Nayak DS, Malode SJ, Kulkarni RM (2017) An electrochemical sensor for clozapine at ruthenium doped TiO₂ nanoparticles modified electrode. *Sensors Actuators B Chem* 247:858–867. <https://doi.org/10.1016/j.snb.2017.03.102>
 35. Xu, Xue; Li, Mengzhi; Hu, Jun; Chen, Zheng; Yu, Jinyu; Dong, Yan; Sun, Chengtao; Han J (2018) Somatic mitochondrial DNA D-loop mutations in meningioma discovered : a preliminary data a comprehensive overview of mitochondrial DNA 4977-bp. *J Cancer Res Ther* 14:1525–1534. <https://doi.org/10.4103/jcrt.JCRT>

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