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Human Papilloma Virus DNA-biomarker Analysis for Cervical Cancer: Signal Enhancement by Gold Nanoparticle-coupled Tetravalent Streptavidin-Biotin Strategy

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Abstract

Human papillomavirus (HPV) is a double-standard DNA virus, as well as the source of infection to the mucous membrane. It is a sexually transmitted disease that brings the changes in the cervix cells. Oncogenes, E6 and E7 play a pivotal role in the HPV infection. Identifying these genes to detect HPV strains, especially a prevalent HPV16 strain, will bring a great impact. Among different sensing strategies for pathogens, the dielectric electrochemical biosensor shows the potential due to its higher sensitivity. In this research, HPV16-E7 DNA sequence was detected on the carbodiimidazole-modified interdigitated electrode (IDE) surface with the detection limit of 1 fM. To enhance the sensitivity, the target sequence was conjugated on gold nanoparticle (GNP) and attained detection to the level of 10 aM. This produced ~100 folds improvement in detecting HPV16-E7 gene and 4 folds increment in the current flow. The stability of HPV16-E7 DNA sequences on GNP was verified by the salt-induced GNP aggregation. The current system has shown the higher specificity by comparing against non-complementary and triple-mismatched DNA sequences of HPV16-E7. This demonstration in detecting HPV16-E7 using dielectric IDE sensing system with a higher sensitivity can be recommended for detecting a wide range of disease-causing DNA-markers.

Keywords: Human papillomavirus; Interdigitated Electrode; Gold nanoparticle; DNA-sensor; Cervical cancer

1. Introduction

Human papillomavirus (HPV) is a common sexually transmitted pathogen and more than 40 HPV strains were found to infect the genital portion of women and men [1]. It is well known and widely accepted that almost all cervical cancers are directly linked to one or more oncogenic type of HPV strains [2,3]. It is wise to identify the HPV infection at earlier stage in order to avoid spreading HPV to other parts of the body. Various strategies and biosensors were shown to detect HPV at a lower detection level. Determining detection limit is highly depending on the selection of suitable biomarker for the desired disease and the surface functionalization chemistry on the sensor. In general, antibody, aptamer, DNA, and RNA were the potential probe to diagnose the HPV biomarkers. With different probes, DNA is the well-established one for detecting HPV and yields a higher sensitive detection. In the current study, to detect HPV we have chosen the capture and detection DNA sequences from E7 gene of HPV strain 16 (HPV16). HPV16 and 18 are the highly

reported strains found to cause severe illness with the cancer, as they have the predominant oncogenes, E6 and E7 [4]. These oncogenes are considered to be vital, due to their interference with the protective proteins p53 and pRb, respectively. Among E6 and E7, the gene encoding E7 protein causes the higher risk HPV, which includes HPV16 and 18. We have desired to use the specific target sequence from E7 of HPV strain 16 (HPV16-E7) and detected by interdigitated electrodes (IDE).

DNA-based sensor plays a major role in the field of medical diagnosis with the accurate DNA hybridization. Involves in the processes of disease and mutated gene identification, drug discovery, analysis with food technology and forensics. DNA hybridization analysis with the help of electrochemical biosensor brings out the above applications with advantages such as, rapid diagnosis, compatibility, high sensitivity, low-cost, portability, and high accuracy [4]. Even though electrochemical DNA sensing system uses different chemistries, they avail the merit of the nanoscale interactions with the analyte in the test sample, the electrode surface and the recognition layer. These potentials have opened the development of ideal signal transducers for the analysis of DNA hybridization with an inexpensive way. DNA hybridization analysis accomplished electrochemically by using different strategies that utilize chemical reactions [26], such as immobilization of the desired enzyme on the electrode [27], catalytic oxidation of DNA, electrochemistry with nanoparticles [32–35], conducting polymers [35–37], and quantum dots. In this line, micro- and nano-fabricated sensor surface have been used to quantify and detect the biomolecules [5,6]. The distance between the electrodes in the interdigitated electrode (IDE) surface plays a crucial role in generating the sensitivity. However, narrowing the gaps between the electrodes to the lower is causing other issues,

such as short-circuit. Instead, nanoparticle-modified IDE sensor surface may aid to reduce the distance between the electrodes and improve the detection.

Different ranges of nanoparticle have been utilized in the biosensing application, these particles include gold, platinum, copper and silver [7–11]. Among different metallic and non-metallic particles, gold nanoparticles (GNPs) have a great attention in the field of biosensor [12,13], in general GNP has been used in two ways with biosensors to improve the sensitivity. One is with the surface modification and the other one is improvement with the gold-conjugated target/capture molecule [14–17]. It was proved that higher number of biomolecules with the proper orientation on the sensing surface drastically enhances the detection range. Higher number of biomolecular immobilization has been demonstrated by using different gold nanostructures and improved the sensitivity [18,19]. To obtain this possibility, antibody or aptamer or DNA can be immobilized on the surface of GNP through electrostatic interaction or chemical modification or physical adsorption [20,21]. With these strategies more numbers of probe are immobilized on the sensing surfaces and created a spatial arrangement for the biomolecular interaction with the proper current flow enhancement to several folds [22]. Gopinath et al. used aptamer conjugated GNP for colorimetric detection of important biomolecules [23]. Lakshmipriya et al. used streptavidin-conjugated GNP to improve the detection of clotting protein factor IX (FIX) and reached the sensitivity of 100 pM [14]. Various sensors have been demonstrated in different ways with GNP for biosensing applications [13,15,24,25]. In this research, we conjugated the target with GNP and performed the sensitive detection on IDE sensor surface to identify HPV16. Two strategies were implemented to improve the detection of HPV16-E7. The first one is the tetravalent streptavidin-biotin strategy. It has been proven that biotin and

streptavidin have the strong binding affinity and works well in several sensors to enhance the sensitivity. In this case, streptavidin has four binding sites, when attach the probe with biotin there is a possibility of capturing higher molecules on the sensing surface through the streptavidin-biotin strategy. Second one is the conjugation of target sequence with GNP leads to the lower limit of detection. Here, combined these two strategies to detect the target sequence of HPV16-E7 and brought the novelty.

2. Materials and Methods

2.1. Reagents and biomolecules

1,1′-Carbonyldiimidazole (CDI) Phosphate Buffer Solution (PBS), streptavidin and gold nanoparticle (GNP) were procured from Sigma-Aldrich (USA). Ethanolamine was bought from Fisher Scientific (UK**)**. Sequences for HPV16-E7 probe [5'-biotin/thiol-C6-ACC ATC TAT TTC ATC CTC CTC CTC-3'] and HPV16-E7 target [5'-GAG GAG GAG GAT GAA ATA GAT GGT-3'] were synthesised by the local commercial supplier. These sequences were originally adapted from the previous study [26].

2.2. IDE sensor surface preparation

IDE fabrication was followed as prepared previously using different parameters with the physical modifications on the surface. Upon completion of the oxidation on silicon wafers at high temperature, the etching process was carried out using the aluminium. After the etching and developing processes on the surface, other procedures were carried out as in the previous study [19]. Eventually, the zinc oxide layer was created on the top. Before being proceeded with the molecular

attachment, the sensing surface was washed by using 1 M potassium hydroxide at pH 9.0.

2.3. Immobilization of target on GNP surface and the stability assay

Prior to performing the surface modification on the IDE sensor surface, the target DNA strand was immobilized on the GNP surface. Different concentrations of the thiolated target strand were mixed independently with 10 µL of GNP (final concentrations were from 1 aM until 10 fM) and kept 30 min at room temperature (RT). The bound GNP with the target was separated by centrifugation (10 min for 10,000 x g). The separated GNP was washed three times by deionized water to remove completely the unbound probe. The final product was kept at 4°C and used for the further analysis. The stability of HPV16-E7 DNA sequence (1 µM) attachment on the GNP was verified by using the salt-induced colorimetric assay. The conjugated DNA probe on GNP was added with 800 mM of NaCl and kept at RT for 30 min. For the control experiment only GNP was added with NaCl in the absence of DNA probe. The visual changes in the solution colour were monitored and the stability of DNA attachment on the GNP was also confirmed by UV-vis spectrometer scanning from 400-800 nm.

2.4. Surface modification of IDE surface to capture HPV16-E7 probe

To capture the target DNA sequence for detecting HPV16-E7 on the IDE sensor surface, the complementary probe DNA sequence was first immobilized through the surface chemistry. Initially, the sensor surface was modified by 1,1′-Carbonyldiimidazole (CDI) to immobilize the streptavidin as the basic molecule. A 0.5 mM of CDI was diluted 30% acetone and dropped on the IDE surface and kept

for 1 h at RT. After that, the surface was washed thoroughly five times with 10 mM PBS (pH 7.4) to remove the unbound CDI. Then, 1 µM of streptavidin was added on the CDI-modified surface followed by blocking was performed with 1 M ethanolamine to cover the remaining CDI surfaces. Following this step, 1 µM of biotinylated capture probe was allowed to interact on the streptavidin-modified surfaces. Finally, 10 fM of target sequence (E7) was added on the surface to detect HPV16.

2.5. Detection of HPV16-E7 on the probe modified IDE surfaces

On the probe-modified surfaces the target sequences was detected in two ways as follows. Method 1 (without GNP): (i) surface was modified by CDI, (ii) 1 µM of streptavidin was immobilized, (iii) the remaining surface was blocked by 1 M ethanolamine, (iv) 1 µM of biotinylated capture probe was added, (v) 10 fM of target sequence was added. In method 2 (with GNP): (i) surface was modified by CDI, (ii) 1 µM of streptavidin was immobilized, (iii) the remaining surface was blocked by 1 M ethanolamine, (iv) 1 µM of biotinylated capture probe was added, (v) 10 fM of target sequence was conjugated with GNP and interacted.

2.6. Comparing the detection limit of HPV16-E7: target vs GNP-target

To evaluate the limit of detection, the HPV16-E7 target sequence was titrated from the lower attomolar to the picomolar range. The target DNA was diluted at the concentrations from 1 aM to 1 pM and dropped independently on the capture probe modified IDE surface. Other experimental steps were followed as stated in the above experiments. For the comparative analysis, similar concentrations using target-GNP were tested on the IDE surface by interacting with the capture probe and the changes in the current were monitored.

2.7. Selective detection of HPV16-E7 on the IDE sensing surface

In this experiment, the mandatory step was taken to evaluate the selective binding of the target with capture probe. For that, the same experiment was performed with the complementary and the triple mis-match sequences instead of the original target sequences. The binding affinities of non-complementary sequences [5-ACC ATC TAT TTC ATC CTC CTC CTC-3] and the triple mis-match sequences [5'-GAG GAG GAG GAT ctt ATA GAT GGT-3] were compared with the target sequences. For this analysis, the same immobilization method was followed to immobilize the capture probe on the IDE surface. Then, 1 pM of complementary or triple mis-match sequences was dropped separately on the surface for the hybridization.

3. Results and Discussion

Human papilloma virus (HPV) is a pathogen, affects the regenerative track, predominantly in the cervix of women. There are different HPV strains identified, among them strains HPV16 and HPV18 cause 70% of precancerous cervical lesions and cervical cancers. Earlier identification of HPV is mandatory to avoid progressing HPV to the next stage. Currently, Pap-test and the visual inspection with acetic acid are in use for identifying HPV strains. The well-developed biosensor is in demand now to detect HPV at an earlier stage and to obtain the better treatment. The good biosensor is expected to have the sensitive and selective detection with a lower amount of analyte. The lower detection level is mainly relying on the interaction of the probe and the target molecule on the properly functionalized surface, in addition, expansion of the surface area helps to capture higher number of molecules. To work

out this notion, this study was carefully chosen the suitable capture probe and the target DNA sequences for the detection of HPV16, with the help of E7 gene sequences. The genuine hybridization of capture and detection DNA sequences was monitored on the chemically-functionalized IDE sensing surface with the aid of electrochemical sensing. To elevate the limit of detection, the target DNA sequence was immobilized on the gold nanoparticle (GNP) surface and detected by hybridizing the capture probe modified IDE surface.

3.1. Tetravalent Streptavidin-biotin Molecular Assembly

The schematic representation for the DNA hybridization with the detection procedure is displayed in figure 1. As shown in the figure, the surface was initially modified by CDI to immobilize the streptavidin. On the streptavidin surface, the biotinylated capture probe was allowed to interact. The binding affinity of biotin-streptavidin is too strong and streptavidin has four binding sites for biotin, ultimately there is a possibility of binding with at least three biotinylated capture probe on one tetravalent streptavidin. By this way, it was managed to immobilize higher number of probe sequences on the sensing surface [13,25]. On the capture probe, two different experiments were performed for the comparison. In the first one, directly added the target DNA sequence and recorded the changes in the current by electrochemical sensing. With the second experimental set-up, the same target DNA sequence was conjugated on GNP and complemented to the probe to monitor the current changes. In other sensing strategies, it has been proven that the gold-conjugated detection enhanced the detection limit to several folds [27]. In the earlier study, streptavidin-antibody-GNP conjugates improved the limit of detection of blood clotting protein (Factor IX) to the picomolar level [14]. In other studies, GNP-aptamer

conjugates were used to enhance the signal against different targets such as, protein [28], DNA [29], bacteria [30], metal ions [31], and cancer biomarkers [32]. In addition, GNP-aptamer based colorimetric assay bring out easier visual detections of the target molecule with the simple steps [33]. Antibody-GNP based colorimetric detection of tuberculosis ESAT-6 was carried out and well-demonstrated with the limit of detection at 1.25 pM [16]. With these evidences, it was expected that with the target conjugated-GNP there will be a room for the improvement in the sensitive detection of HPV16-E7 sequence on the proposed IDE electrochemical sensing surface.

3.2. Surface Chemistry and Biomolecular Assembly

Before being initiate the main experiment, the uniformity of the IDE surface was observed under Scanning Electron Microscopy and it displays the occurrence of prevalent finger and gap arrangements (Figure 2a). On the other hand, the stability of the target HPV16-E7 sequence attached on the GNP was tested in the presence of the monovalent ion (NaCl). It was cleared that the target DNA sequence attached GNP was stable-enough, as it shows an apparent peak maxima at ~520 nm under UV-Vis scanning profile (Figure 2b). Figure 3a shows the evidences obtained on different devices synthesized, indicate the reproducibility with the fabrication. The dipole mechanism is the basic principle with the dielectric sensor, occurs between positive and negative charges is displayed in Figure 3a (inset). As shown in the figure, the bare device shows the current level at $2.57E^{-03}$ and within the background error values compared to other prepared devices. After modification with CDI the current level was decreased to $7.28E^{-06}$, due to the differences in the charge between the original bare surface and CDI-functionalized surface. This change confirms that the surface has been modified properly. After CDI modification, the

surface was surface attached with streptavidin to interact the biotinylated-DNA probe, as shown in figure 3b.

3.3. HPV16-E7 DNA Sequence Complementation

After adding the streptavidin, the current change was noticed from $7.28E^{-06}$ to $8.53\ E^{-06}$. Then the biotinylated-probe was increased the current level to $1.12\ E^{-05}$ upon interaction. This big change in current confirms the genuine interaction of biotin on the streptavidin. When added 10 fM of target sequence on the biotinylated surface the change in the current flow was noted from $1.12\ E^{-05}$ to $8.3\ E^{-06}$. It is important to notice the reason behind when interacted the biotinylated single-stranded probe, there was an increment in the current and decreased when the duplex is formed. These changes in the opposite direction are primarily due to the charge originated from the phosphate backbone on the DNA strands. When there is a single-strand, the negative charge is exposed from the phosphate backbone, whereas upon duplex formation the second strand is masked the phosphate backbone exposure, ultimately the surface is become less negatively charged. This result confirms the proper binding of capture and detection DNA strands on the chemically followed by biologically-modified sensing surface. The detection with the lower level of the target DNA sequences might be achieved mainly through the interaction of streptavidin-biotin.

To improve the limit of detection with HPV16-E7, the target DNA was conjugated with GNP and allowed to bind on the surface immobilized with the capture probe. Figure 4a&b shows the comparison on the detection with the constant concentration (10 fM) of target with and without GNP (Figure 4a). Only the target DNA without GNP shows the current flow change from $1.12\ E^{-05}$ to $8.3\ E^{-06}$. On the other hand, with the same concentration of the target conjugated-GNP, the current

flow was from 1.15 E^{-05} to 2.25 E^{-06} (Figure 4b). This shows ~4 times higher changes in current, and it might be due to the higher number of target binding on the surface of GNP, in addition to the impact of GNP. Previously, it has been proven that streptavidin-conjugated secondary antibody enhanced the detection of ESAT-6 protein by Enzyme-linked Immunosorbant assay and attained the limit of detection to the lower picomolar. In the current study, the capture probe was attached with biotin, ultimately the higher number of capture probe was bound on the IDE surface through the interaction of streptavidin-biotin and increased the binding and detection of target oligo with/without GNP.

3.4. Determination of Limit of Detection

To evaluate the limit of detection precisely, we performed the above interaction with different concentrations (100 aM to 1 pM) of target HPV16-E7 DNA sequences and added on the capture probe modified IDE surface. To enhance the limit of detection, the similar experiment was carried out with the target-conjugated GNP (1 aM to 10 fM). Figure 5a & b shows the comparison on the limit of detection with single-stranded probe against the target DNA with and without GNP conjugation. As displayed in figure 5a, 100 aM concentration is not showing any measurable change in the current flow, however, from 1 fM it started to indicate the current flow with the decrement. Upon increasing the concentration of the target DNA, the current flow decreases further, and the limit of detection was attained to be 1 fM. In the case of target with GNP (Figure 5b), 1 aM concentration did not show any change in the current flow, but from 10 aM it was getting the charge changes with all the subsequent concentrations, and the limit of detection was found as 10 aM. This experiment clearly indicates 100 folds improvement with the detection limit

of HPV16-E7 DNA with GNP conjugation compared to the case without GNP. Moreover, it has been noticed that in the case of the target without GNP, the current changes for each concentration was with a minimal difference, at the same time the target with GNP shows an apparent changes in the current flow with each concentration evaluated. Further, 3σ estimation has been done with the linear plot and confirmed the limit of detection (Figure 6a). This result evidenced the detection of target (HPV16-E7) with the enhanced sensing performance using the above strategy with the tetravalent streptavidin-biotin complexed probe and GNP target conjugation.

3.5. Determination of Specificity: DNA Non-complementation and Triple-mismatch

The specific detection of target complementary DNA sequences was compared with non-complementary and triple-mismatch sequences. The higher concentration (1 pM) detected in the above experiment was chosen for this analysis. As shown in the figure 6b, it was found that the target DNA can specifically match to complementary sequence and displayed the decrement in the current. Whereas, the non-complementary HPV16-E7 DNA sequences do not show any significant change in the current flow, indicates there is no complementation occurred on the immobilized probe DNA. On the other hand, the triple-mismatched DNA sequences displayed a small decrement in the current flow, represents there is a huge disturbance in the complementation. From this result it was clearly proven that the target DNA was specifically detected by the complementary target HPV16-E7 DNA sequences.

4. Conclusion

Human papillomavirus (HPV) is an important pathogen need to be considered for the medical development, HPV affects the skin and the moist membrane, a worldwide potential issue. Different strains of HPV play a critical role in causing the cervical cancer, predominantly with women. The oncogenes, E6 and E7 play a crucial role in causing the HPV infection, and the strains HPV16 and HPV18 have the predominant role. Identifying these genes helps to detect the HPV infection at an earlier stage for the proper remedy. Electrochemical biosensor with the dielectric strategy is shown here with a greater attention to detect by the hybridization using E7 DNA sequences of HPV16. In this investigation, HPV16-E7 capture probe was detected by the target sequence on the CDI-modified interdigitated electrode sensor with the chemical and biological complex assemblies of tetravalent-biotin followed by HPV16-E7 duplex formation. The limit of detection was achieved is 1 fM and enhanced by the GNP conjugation to 100 folds with detection at 10 aM along with the higher voltage differences. GNP-conjugated target detection through the streptavidin-biotin strategy on the interdigitated electrode electrochemical sensor can be followed for other diseases using different probes including DNA as demonstrated here.

References

[1] N. Muñoz, F.X. Bosch, S. de Sanjosé, R. Herrero, X. Castellsagué, K. V. Shah, P.J.F. Snijders, C.J.L.M. Meijer, Epidemiologic Classification of Human Papillomavirus Types Associated with Cervical Cancer, N. Engl. J. Med. 348 (2003) 518–527. doi:10.1056/NEJMoa021641.

[2] E.-K. Yim, J.-S. Park, The Role of HPV E6 and E7 Oncoproteins in HPV-associated Cervical Carcinogenesis, Cancer Res. Treat. 37 (2005) 319. doi:10.4143/crt.2005.37.6.319.

[3] A.L.P. Abreu, R.P. Souza, F. Gimenes, M.E.L. Consolaro, A review of methods for detect human Papillomavirus infection, Virol. J. 9 (2012). doi:10.1186/1743-

422X-9-262.

[4] E. Rasouli, Z. Shahnavaz, W.J. Basirun, M. Rezayi, A. Avan, M. Ghayour-Mobarhan, R. Khandanlou, M.R. Johan, Advancements in electrochemical DNA sensor for detection of human papilloma virus - A review, Anal. Biochem. 556 (2018) 136–144. doi:10.1016/j.ab.2018.07.002.

[5] O.C. Cheen, S.C.B. Gopinath, V. Perumal, M.K.M. Arshad, T. Lakshmipriya, Y. Chen, R. Haarindraprasad, B.S. Rao, U. Hashim, K. Pandian, 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 (2017) 117–125. doi:10.1007/s00604-016-2001-6.

[6] S.C.B. Gopinath, V. Perumal, R. Kumaresan, T. Lakshmipriya, H. Rajintraprasad, B.S. Rao, M.K.M. Arshad, Y. Chen, N. Kotani, U. Hashim, Nanogapped impedimetric immunosensor for the detection of 16 kDa heat shock protein against Mycobacterium tuberculosis, Microchim. Acta. (2016). doi:10.1007/s00604-016-1911-7.

[7] N.Z. Jin, S. Anniebell, S.C.B. Gopinath, Y. Chen, Variations in Spontaneous Assembly and Disassembly of Molecules on Unmodified Gold Nanoparticles, Nanoscale Res. Lett. 11 (2016) 399. doi:10.1186/s11671-016-1615-2.

[8] N.S. Ismail, S.C.B. Gopinath, Enhanced antibacterial effect by antibiotic loaded starch nanoparticle, J. Assoc. Arab Univ. Basic Appl. Sci. 24 (2017) 136–140. doi:10.1016/j.jaubas.2016.10.005.

[9] S. Anniebell, S.C.B. Gopinath, Polymer Conjugated Gold Nanoparticles in Biomedical Applications, (2018) 1–13. doi:10.2174/0929867324666170116123633.

[10] P. Velusamy, C.-H. Su, G. Venkat Kumar, S. Adhikary, K. Pandian, S.C.B. Gopinath, Y. Chen, P. Anbu, Biopolymers Regulate Silver Nanoparticle under Microwave Irradiation for Effective Antibacterial and Antibiofilm Activities, PLoS One. 11 (2016) e0157612. doi:10.1371/journal.pone.0157612.

[11] S.C.B. Gopinath, C.S. Goh, M. Citartan, T. Lakshmipriya, M.K. Arshad, M.F. Fatin, A.R. Ruslinda, U. Hashim, S. V Chinni, Micro-Encapsulation of Antibiotic in Cellulose Nanoparticle Inhibits Bac- teria, Micro Nanosyst. (2016) 1–6. doi:10.2174/1876402908666160720104O.

[12] S.C.B. Gopinath, T. Lakshmipriya, K. Awazu, Colorimetric detection of controlled assembly and disassembly of aptamers on unmodified gold nanoparticles, Biosens. Bioelectron. 51 (2014) 115–123. doi:10.1016/j.bios.2013.07.037.

[13] T. Lakshmipriya, S.C.B. Gopinath, T.-H. Tang, Biotin-Streptavidin Competition Mediates Sensitive Detection of Biomolecules in Enzyme Linked Immunosorbent Assay, PLoS One. 11 (2016) e0151153. doi:10.1371/journal.pone.0151153.

[14] T. Lakshmipriya, M. Fujimaki, S.C.B. Gopinath, K. Awazu, Y. Horiguchi, Y. Nagasaki, A high-performance waveguide-mode biosensor for detection of factor IX using PEG-based blocking agents to suppress non-specific binding and improve sensitivity, Analyst. 138 (2013) 2863. doi:10.1039/c3an00298e.

[15] T. Lakshmipriya, Y. Horiguchi, Y. Nagasaki, 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 (2014) 3977–85. doi:10.1039/c4an00168k.

[16] F. Wang, T. Lakshmipriya, S.C.B. Gopinath, Red Spectral Shift in Sensitive Colorimetric Detection of Tuberculosis by ESAT-6 Antigen-Antibody Complex : a New Strategy with Gold Nanoparticle, Nanoscale Res. Lett. (2018) 1–8.

[17] S.C.B. Gopinath, R. Kumaresan, K. Awazu, M. Fujimaki, M. Mizuhata, J. Tominaga, P.K.R. Kumar, Evaluation of nucleic acid duplex formation on gold over layers in biosensor fabricated using Czochralski-grown single-crystal silicon substrate, Anal. Bioanal. Chem. 398 (2010) 751–758. doi:10.1007/s00216-010-3994-z.

[18] I. Letchumanan, M.K. Md Arshad, S.R. Balakrishnan, S.C.B. Gopinath, Gold-nanorod enhances dielectric voltammetry detection of c-reactive protein: A predictive strategy for cardiac failure, Biosens. Bioelectron. 130 (2019) 40–47. doi:10.1016/j.bios.2019.01.042.

[19] I. Letchumanan, S.C.B. Gopinath, M.K. Md Arshad, P. Anbu, T. Lakshmipriya, Gold nano-urchin integrated label-free amperometric aptasensing human blood clotting factor IX: A prognosticative approach for “Royal disease,” Biosens. Bioelectron. 131 (2019) 128–135. doi:10.1016/j.bios.2019.02.006.

[20] C.M. Niemeyer, B. Ceyhan, P. Hazarika, Oligofunctional DNA-Gold Nanoparticle Conjugates, Angew. Chemie - Int. Ed. 42 (2003) 5766–5770. doi:10.1002/anie.200352744.

[21] T.A. Taton, Preparation of Gold Nanoparticle-DNA Conjugates, in: Curr. Protoc. Nucleic Acid Chem., 2002: p. 12.2.1-12.2.12. doi:10.1002/0471142700.nc1202s09.

[22] V. Perumal, M. Shuaib, M. Saheed, N. Muti, J. Chiu, Biosensors and Bioelectronics Gold nanorod embedded novel 3D graphene nanocomposite for selective bio-capture in rapid detection of Mycobacterium tuberculosis, Biosens. Bioelectron. 116 (2018) 116–122. doi:10.1016/j.bios.2018.05.042.

[23] S.C.B. Gopinath, T. Lakshmipriya, K. Awazu, Colorimetric detection of controlled assembly and disassembly of aptamers on unmodified gold nanoparticles, Biosens. Bioelectron. 51 (2014) 115–123. doi:10.1016/j.bios.2013.07.037.

[24] S.C.B. Gopinath, K. Awazu, M. Fujimaki, K. Shimizu, T. Shima, Observations of Immuno-Gold Conjugates on Influenza Viruses Using Waveguide-Mode Sensors, PLoS One. 8 (2013) 1–10. doi:10.1371/journal.pone.0069121.

[25] T. Lakshmipriya, S.C.B. Gopinath, M. Citartan, U. Hashim, T.-H. Tang, Gold Nanoparticle-mediated high-performance enzyme-linked immunosorbent assay for detection of tuberculosis ESAT-6 protein, Micro Nanosyst. 8 (2016) 92–98. doi:10.2174/1876402908666161026154223.

[26] L. Civit, A. Fragoso, C.K. O’Sullivan, Electrochemical biosensor for the multiplexed detection of human papillomavirus genes, Biosens. Bioelectron. (2010). doi:10.1016/j.bios.2010.06.072.

[27] T. Lakshmipriya, Y. Horiguchi, Y. Nagasaki, 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 (2014) 3977–3985. doi:10.1039/c4an00168k.

[28] H. Pandana, K.H. Aschenbach, R.D. Gomez, Systematic aptamer-gold nanoparticle colorimetry for protein detection: Thrombin, IEEE Sens. J. 8 (2008) 661–666. doi:10.1109/JSEN.2008.922725.

[29] H. Tao, L. Wei, A. Liang, J. Li, Z. Jiang, H. Jiang, Highly sensitive resonance scattering detection of DNA hybridization using aptamer-modified gold nanopaticle as catalyst, Plasmonics. 5 (2010) 189–198. doi:10.1007/s11468-010-9133-z.

[30] X. Ma, Y. Liu, N. Zhou, N. Duan, S. Wu, Z. Wang, SERS aptasensor detection of: Salmonella typhimurium using a magnetic gold nanoparticle and gold nanoparticle based sandwich structure, Anal. Methods. 8 (2016) 8099–8105. doi:10.1039/c6ay02623k.

[31] J.F. Bao, P. He, X.J. Yu, S.L. Jiang, Q.Y. Yan, Detection of Hg2+ Using Gold Nanoparticle Probes, Acta Chim. Sin. 67 (2009) 2139–2143.

[32] C.D. Medley, J.E. Smith, Z. Tang, Y. Wu, S. Bamrungsap, W. Tan, Gold nanoparticle-based colorimetric assay for the direct detection of cancerous cells, Anal. Chem. 80 (2008) 1067–1072. doi:10.1021/ac702037y.

[33] J.C. Liu, Z.Z. Lu, A.L. Chen, Gold Nanoparticle-Aptamer Based Colorimetric Biosensing Assays, Spectrosc. Spectr. Anal. 34 (2014) 2040–2046. doi:10.3964/j.issn.1000-0593(2014)08-2040-07.

Figure legends

Figure 1: Schematic representation on detection of HPV16-E7 by GNP-conjugated target for the signal enhancement. The E7 DNA sequences are shown.

Figure 2: (a) SEM image of IDE sensing surface; (b) Stability of GNP and probe conjugation under UV-Vis spectroscopy. GNP-probe conjugation shows the dispersion with the red color solution and the absorption maxima is at 520 nm. The control with only GNP shows the aggregation with purple color solution and shifted the spectrum to ~600 nm.

Figure 3: (a) Measurement with the current level using three different preparations of IDE sensing surface. Showing the similar levels of current flow and figure inset displays the mechanism behind. (b) Detection of 1 pM target DNA on IDE surface. The spectra for surface modifications are included.

Figure 4: Comparison on detection of 1 pM target DNA. (a) target DNA alone; (b) target conjugated GNP. Arrows indicate the direction of changes.

Figure 5: Limit of detection of HPV16 target DNA. (a) target DNA concentrations from 100 aM to 1 pM; (b) target DNA-GNP concentrations from 1 aM to 10 fM. The concomitant changes in the spectra can be seen.

Figure 6: (a) Limit of detection of target DNA by linear graph. (b) Specificity analysis. Carried out with non-complementary and triple mismatch sequences and compared with complementary sequences. Averaged values are displayed.

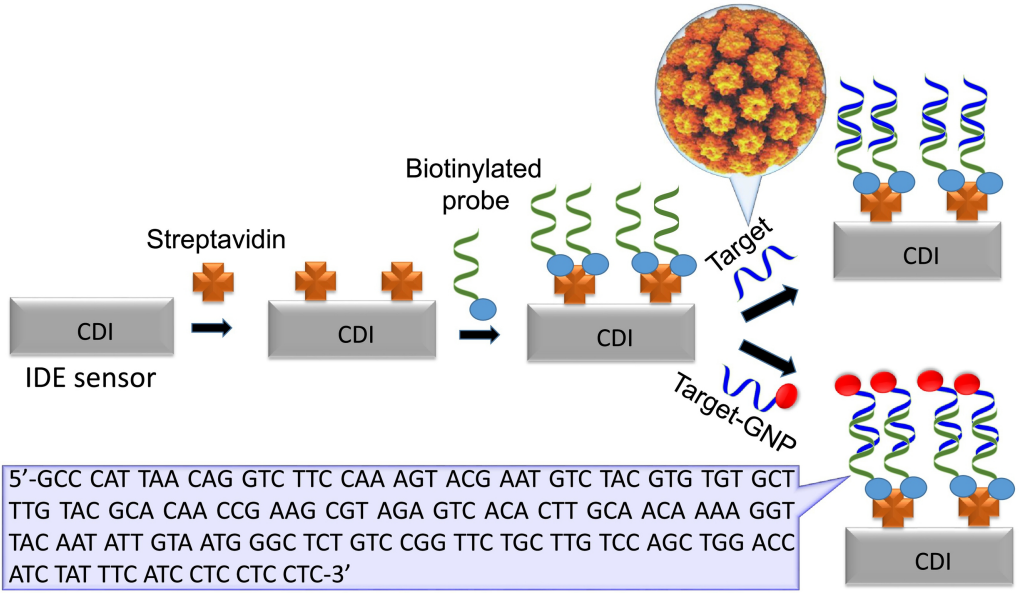
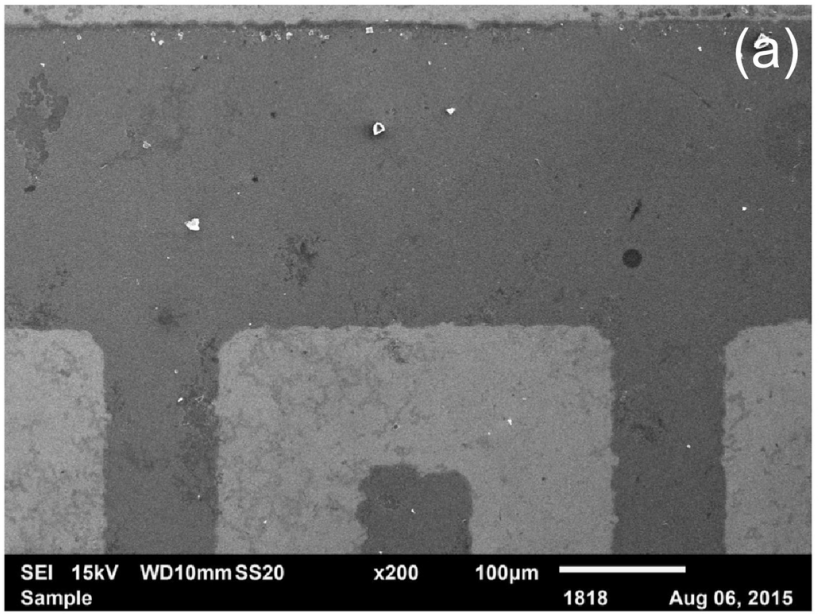

Biotinylated
probe
Streptavidin
Target
CDI
CDI
CDI
CDI
CDI
IDE sensor
Target-GNP
5’-GCC CAT TAA CAG GTC TTC CAA AGT ACG AAT GTC TAC GTG TGT GCT
TTG TAC GCA CAA CCG AAG CGT AGA GTC ACA CTT GCA ACA AAA GGT
TAC AAT ATT GTA ATG GGC TCT GTC CGG TTC TGC TTG TCC AGC TGG ACC
ATC TAT TTC ATC CTC CTC CTC-3’

Figure 1


(a)
SEI 15kV WD10mm SS20 x200 100µm
Sample 1818 Aug 06, 2015

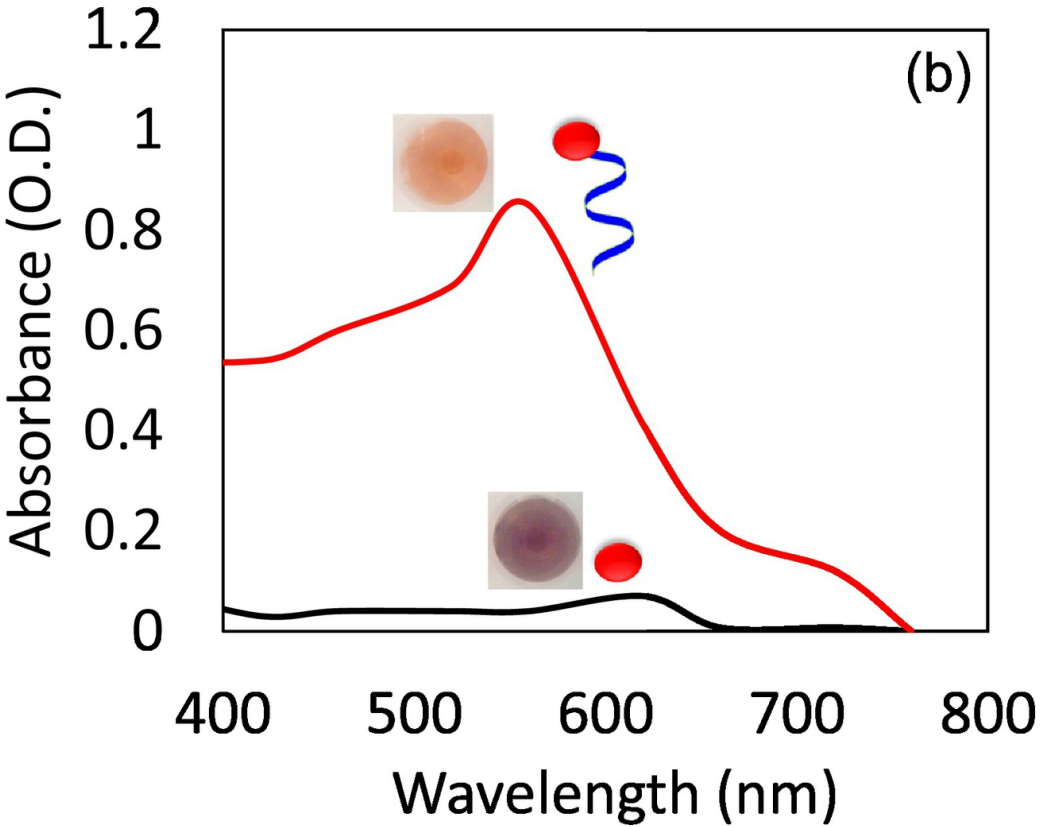

(b)
Absorbance (O.D.)
1.2
1
0.8
0.6
0.4
0.2
0
400
500
600
700
800
Wavelength (nm)

Figure 2

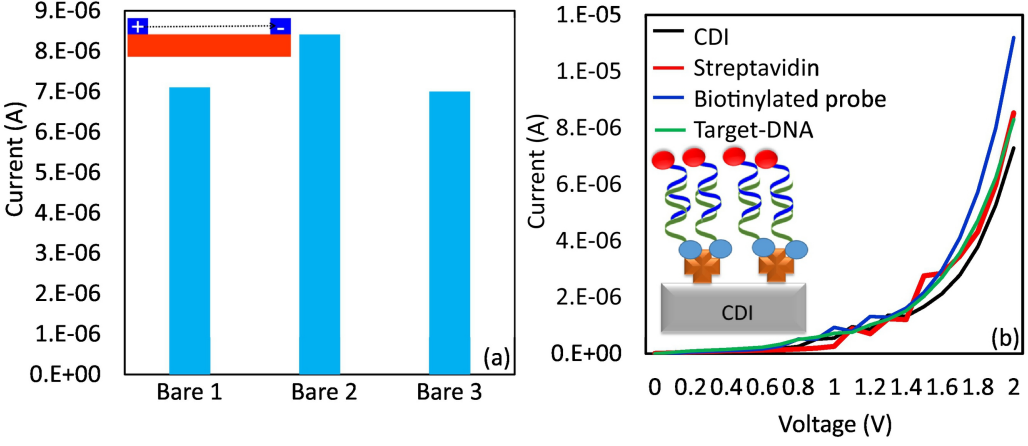

9.E-06
8.E-06
7.E-06
6.E-06
5.E-06
4.E-06
3.E-06
2.E-06
1.E-06
0.E+00
Current (A)
+
-
Bare 1
Bare 2
Bare 3
(a)
1.E-05
1.E-05
8.E-06
6.E-06
4.E-06
2.E-06
0.E+00
Current (A)
CDI
Streptavidin
Biotinylated probe
Target-DNA
CDI
(b)
0 0.2 0.4 0.6 0.8 1 1.2 1.4 1.6 1.8 2
Voltage (V)

Figure 3

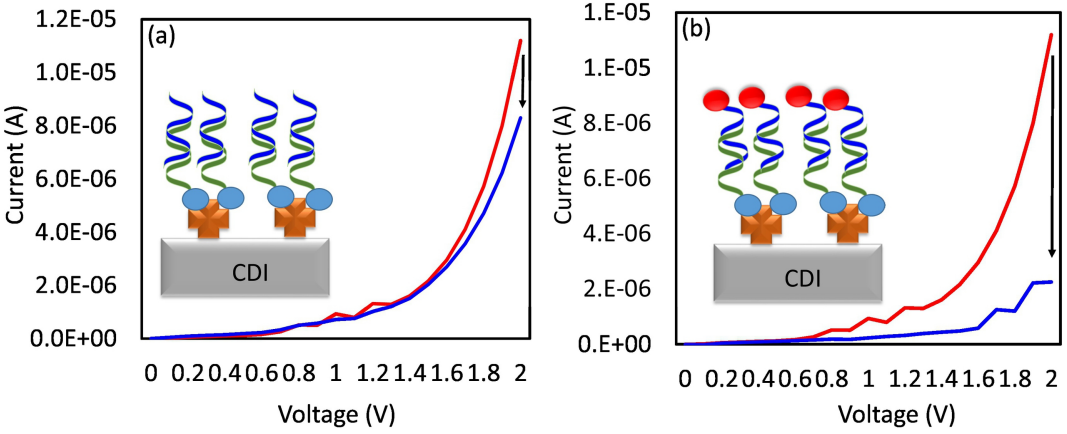

(a)
1.2E-05
1.0E-05
8.0E-06
6.0E-06
4.0E-06
2.0E-06
0.0E+00
Current (A)
CDI
0 0.2 0.4 0.6 0.8 1 1.2 1.4 1.6 1.8 2
Voltage (V)
(b)
1.E-05
1.E-05
8.E-06
6.E-06
4.E-06
2.E-06
0.E+00
Current (A)
CDI
0 0.2 0.4 0.6 0.8 1 1.2 1.4 1.6 1.8 2
Voltage (V)

Figure 4

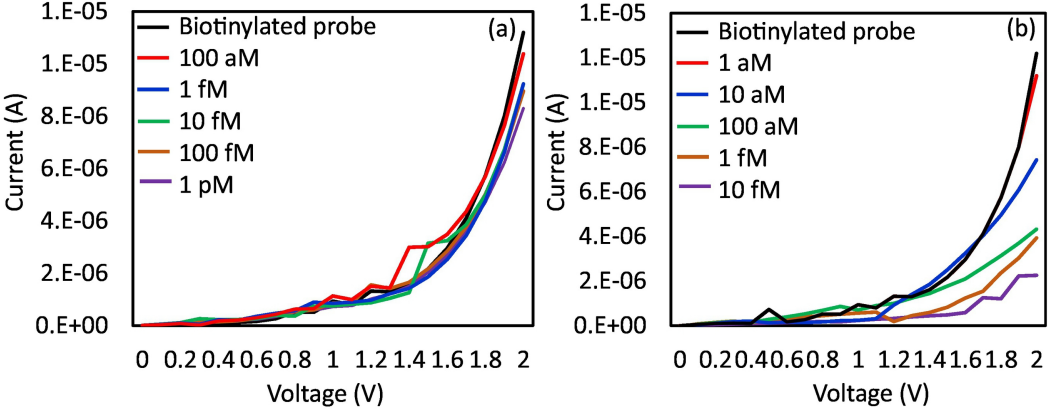

(a)
Biotinylated probe
100 aM
1 fM
10 fM
100 fM
1 pM
Current (A)
1.E-05
1.E-05
8.E-06
6.E-06
4.E-06
2.E-06
0.E+00
0 0.2 0.4 0.6 0.8 1 1.2 1.4 1.6 1.8 2
Voltage (V)
(b)
Biotinylated probe
1 aM
10 aM
100 aM
1 fM
10 fM
Current (A)
1.E-05
1.E-05
8.E-06
6.E-06
4.E-06
2.E-06
0.E+00
0 0.2 0.4 0.6 0.8 1 1.2 1.4 1.6 1.8 2
Voltage (V)

Figure 5

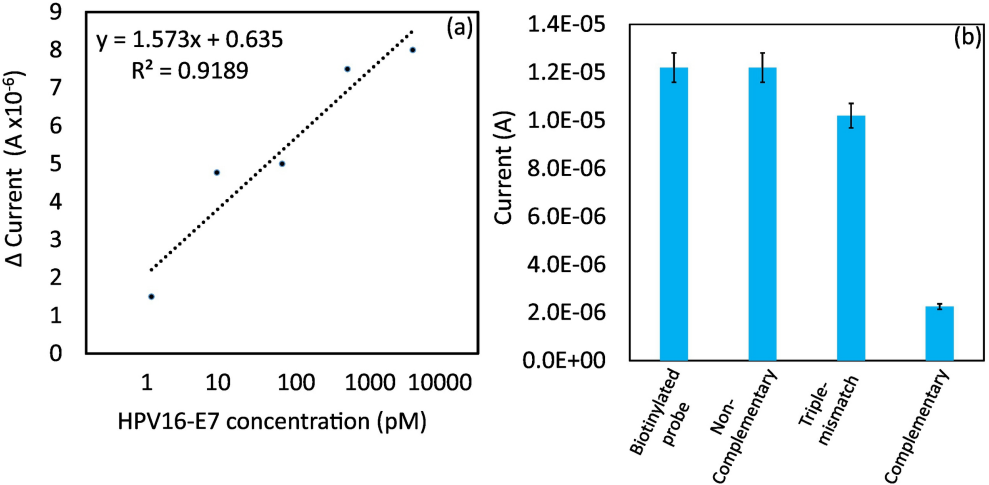

(a)
y = 1.573x + 0.635
R² = 0.9189
Δ Current (A x10-6)
HPV16-E7 concentration (pM)
1
10
100
1000
10000
(b)
Current (A)
1.4E-05
1.2E-05
1.0E-05
8.0E-06
6.0E-06
4.0E-06
2.0E-06
0.0E+00
Biotinylated probe
Non-Complementary
Triple-mismatch
Complementary

Figure 6