Gold Nano-morphology Implemented Voltammetry Immunosensing for Factor IX: In Advance Procedure for 'Christmas Disease'

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ABSTRACT: This work demonstrates the comparative study between existing research work utilizing novel gold nanoparticles and the current work utilizing different gold nano-morphology for the detection of factor IX (FIX) antigen as the early precautionary step. Herein, an interdigitated electrode (IDE) was used by modifying the surface with anti-FIX aptamer as a probe to bind FIX antigen. Surface topography analysis was carried out using scanning electron microscopy (SEM), atomic force microscopy (AFM) and high power microscopy (HPM). Electrical characterization was done using picoammeter with 0V to 2V of linear sweep.

Keywords: *Gold nanoparticles; FIX; IDE.*

1. INTRODUCTION

Tremendous integration of nanotechnology was initiated from the electronics industry for the development of unique sensors on silicon chips [1]. Zubiate et al. reported an integration of nanomaterials in the biosensor that can improve the sensitivity [2] and the related biosensing applications. Among all types of nanomaterials, the highlighted nanomaterial for the construction of robust and efficient biosensor was gold nanoparticle (GNPs) due to their easy production and easier conjugation with biomolecules [3]. GNPs combine high surface area and surface energy [4] with high conductivity [5] and offers a large number of functional groups on the surfaces, enabling particles to be easily functionalized by different types of biomolecules or covalently attached to the electrode [6].

Haemophilia is blood clotting disease due to the lacking the level of FIX protein, also known as blood circulating biomarker. 'Christmas disease' or 'Royal disease' is commonly engaged with the deficiency of blood clotting factor, FIX. Human blood clotting mechanism occur when thrombin catalyzes the conversion of fibrinogen into fibrin [7]. This will obviously contribute to the mesh of fibrin network which manages to plug the gap in the ruptured area. Albeit, the insufficient concentration or decrease in the activity of several clotting factors may cause hemophilia, especially with the early clotting stage FIX. Hence, a better detection strategy is highly recommended for the accurate and early clinical results. Thus, a comparative study was conducted using two different gold nano morphology.

2. METHODOLOGY

Initially, the sensing surface of IDE was rinsed with 70 % ethanol to eliminate the foreign particles then blow dried. In order to create the chemical linkage between sensing surface and biomolecules, an active surface area of IDE was treated with 0.5 M carbodiimide and left of 1 hour at room temperature. Later, after the incubation the surface was cleaned using 10 mM PBS (pH 7.4) to remove the unbound molecules. Herein, carboxyl modified gold nano urchin was pre-mixed with water soluble 1-Ethyl-3- (3-dimethylaminopropyl) carbodiimide (EDC) and active ester compounds N-hydroxy-succinimide (NHS). This mixture was injected on the IDE surface. Then, the chemically modified sensing surface was treated with anti-FIX aptamer with fixed concentration of 1 µM and incubated for 30 minutes followed by washing steps using PBS. Blocking agent was utilized to fill up the unbound spaces in order to avoid non-specific binding. Herein, 1 M of ethanolamine was passed on the sensing surface and leave for 1 hour followed by washing steps. Later, different concentrations of FIX antigen was titrated independently on the IDE surface for detection purpose. Electrical characterization was carried out to study the effective binding event between the biomolecules using Keithley 6487 picoammeter. Similar procedure was carried out using different gold nanostructure, which is gold nano rod. Surface morphology analysis was done using different microscopic methods. For examples High Power Microscopy (HPM), 3D Nanoprofilometer and Atomic Force Microscopy (AFM).

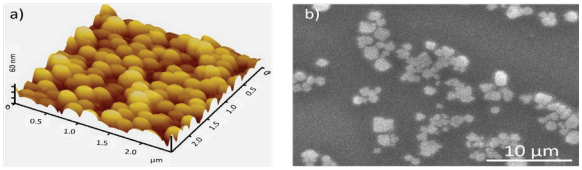

Figure 1: Gold nano-urchin imaging

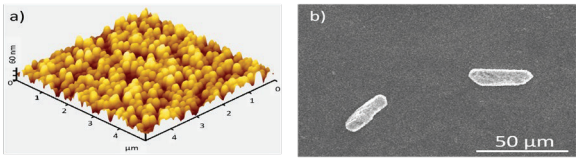

Figure 2: Gold nano rod imaging

The morphology of the different gold nano structures was apparently analyzed under higher magnification using Atomic Force Microscope (AFM) and Scanning Electron Microscope (SEM). Uniform distribution of gold nano urchin can be seen in AFM

analysis (Figure 1a). Herein, the spiking out like structure of gold nanourchin can be seen using SEM analysis (Figure 1b). Similar analysis method was used to carry out surface morphology analysis of gold nanorod. AFM analysis was done to display the outstanding size distribution of gold nano rod (Figure 2a). SEM analysis clearly illustrates the view of elongated gold nano rod (Figure 2b).

3. RESULTS AND DISCUSSION

Two different case studies were conducted in this work in order to compare the existing results in research market [8,9]. First study was conducted using gold nano-urchin, while the second study was conducted using gold nano-rod. The voltammetry results for both cases justify that gold nano-morphology has better sensing performance compared with novel gold nanoparticles. Figures 3 and 4 shows the current increment as the concentrations of FIX increases. For the first case, it was using gold nano-urchin (Figure 3) the current reading for 100 fM while for the second case using gold nano-rod (Figure 4), the current reading for 10 fM is higher than the current reading for anti-FIX aptamer. Thus, the sensitivity levels begin at 100 fM for first case study, while 10 fM for the second study. The current reading increases as the concentration of sample increases. Such behaviour occurs because there was a large volume of biomolecular activity on the sensor gap, the resistance to direct current flow on the electrode surface increases, which results in increment of resistance gradually. The current flow will be even until the molecules were connected, without any barriers. The creation of a complex molecular structure induced vibrations, which triggered the displacement of the charge at the dielectric distance. Generated displacement of charge with a solid confinement called 'dipole moment'.

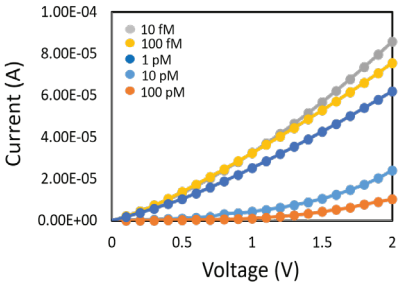

Figure 3: Electrical measurements for different FIX concentrations detection using gold nano-urchin.

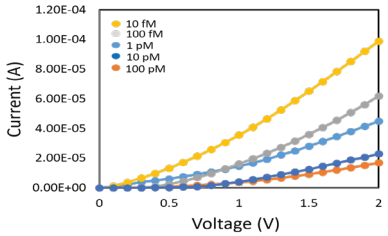

Figure 4: Electrical measurements for different FIX concentrations detection using gold nano-rod.

4. CONCLUSION

This study concludes that gold nano-morphology is an outstanding element for the better sensing performance. As compared with previous studies, herein in this work both case study reveals higher sensitivity level as 100 fM for gold nano-urchin and 10 fM for gold nano-rod with the implemented detection strategy. Hence, strategy proposed in this study is cost effective and reliable to be implemented for healthcare department.

REFERENCES

[1] D. P. Nikolelis and G. P. Nikoleli, "Nanotechnology and biosensors," *Nanotechnol. Biosens.*, vol. 22, pp. 1–446, 2018.

[2] P. Zubiate, C. R. Zamarreño, P. Sánchez, I. R. Matias, and F. J. Arregui, "High sensitive and selective C-reactive protein detection by means of lossy mode resonance based optical fiber devices," *Biosens. Bioelectron.*, vol. 93, pp. 176–181, 2017.

[3] C. Daruich De Souza, B. Ribeiro Nogueira, and M. E. C. M. Rostelato, "Review of the methodologies used in the synthesis gold nanoparticles by chemical reduction," *J. Alloys Compd.*, vol. 798, pp. 714–740, 2019.

[4] S. C. B. Gopinath, T. Lakshmipriya, M. K. Md Arshad, M. N. A. Uda, and Y. Al-Douri, *Nanoelectronics in Biosensing Applications*. Elsevier Inc., 2019.

[5] I. Letchumanan, M. K. Md Arshad, S. R. Balakrishnan, and S. C. B. Gopinath, "Gold-nanorod enhances dielectric voltammetry detection of c-reactive protein: A predictive strategy for cardiac failure," *Biosens. Bioelectron.*, vol. 130, no. October 2018, pp. 40–47, 2019.

[6] L. A. Dykman and N. G. Khlebtsov, "Gold nanoparticles in chemo-, immuno-, and combined therapy: review [Invited]," *Biomed. Opt. Express*, vol. 10, no. 7, p. 3152, 2019.

[7] S. C. B. Gopinath, D. Balasundaresan, J. Akitomi, and H. Mizuno, "An RNA aptamer that discriminates bovine factor IX from human factor IX," *J. Biochem.*, vol. 140, no. 5, pp. 667–676, 2006.

[8] S.C.B. Gopinath, V. Perumal, S.R. Balakrishnan, M.K. Md Arshad, T. Lakshmipriya, C.H. Voon, R. Haarindraprasad, T. Vijayakumar, Chen, Y., U. Hashim, "Voltammetric immunoassay for the human blood clotting factor IX by using nanogapped dielectrode junctions modified with gold nanoparticle-conjugated antibody," *Microchim. Acta*, vol. 184, no. 10, pp. 3739–3745, 2017.

[9] O.C. Cheen, S.C.B. Gopinath, V. Perumal, M.K. Md Arshad, T. Lakshmipriya, Y. Chen, R. Haarindraprasad, S.R. Balakrishnan, 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," *Microchimica Acta* vol. 184, pp. 117–125, 2017.