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RESEARCH ARTICLE

Au nanorods/ g-C_3N_4 composite based biosensor for electrochemical detection of chronic lymphocytic leukemia

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ABSTRACT

Objective(s): With the increasing incidence of cancer and the dramatic effect of early detection on treatment and increase patient's life, many efforts have been devoted to making sensitive diagnosis systems. DNA as a biomarker for diagnosis of different types of cancers at the early stages of illness has attracted much attention.

Methods: In this research novel electrochemical biosensor was developed using titanium phosphate nanoparticles which modified with Pb^{2+} and two DNA as capture probes. Considerable amounts of lead ions were mounted on the surface of titanium phosphate which produced the electrochemical signal. The surface of the biosensor electrode was modified by Au nanorods/ g-C_3N_4 composite. The functional group on the surface of g-C_3N_4, the chemical composition of tip, the morphology of composite and elemental composition of the composite were investigated by Fourier Transform Infrared Spectroscopy, X-Ray Diffraction, Field Emission Scanning Electron Microscope, Energy Dispersive X-Ray Spectroscopy, respectively.

Results: The peaks of C, N, and Au were observed in the EDS spectrum. The presence of Au peaks in the EDS spectrum confirmed the formation of composite from Au nanorods and g-C_3N_4 sheets. Whit this biosensor detection limit of 20 PM and the linear range from 0.6 nM to 6.4 nM for target DNA was obtained.

Conclusions: Finally, it seems that Au-nanorods/g-C_3N_4 composite modified glassy carbon electrode is a good candidate for cancer diagnosis in the early stages.

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INTRODUCTION

One of the most important researches in medical science is the cancer study. Largely cancer affection arises from a mutation in genes. One of the most common leukemia is Chronic lymphocytic leukemia (CLL) which in adults causes an increase in the number of white blood cells called B lymphocytes in the bone marrow. B lymphocytes contribute make antibodies to fight disease. The cancerous cells outspread from the bone marrow to blood and can influence the lymph nodes or liver and spleen. CLL results in low blood counts and weakens the immune system [1]. The rate of CLL incidence is increasing [2]. The key features to determine the therapeutic regime for a patient are early, efficient and specification detection of cancer illness [3]. The biosensor as a new and noninvasive analytical instrument to monitor genomic mutation provides promising advances in research about cancer [4]. Using nanomaterials for the fabrication of biosensors to detect low cancer cell count increase the surface area for

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Table 1. sequences of target DNA and capture DNA1 and capture DNA2

name	sequences
Target DNA	5′-ACGTTATGCGTGCTGGCGCAGGG-3′
Capture DNA1	5′-SH(CH_2)$_6$-CCCTGCGCCAGC-3′
Capture DNA2	5′- ACGCATAACGT -Biotin-3′

maximum detection. Gold nanoparticles are good candidates to prepare biosensors due to they can supply a stable biomolecules immobilization that retains their bioactivity [5, 6]. Gold nanomaterials have biocompatibility, high stability, and simple preparation. The surface chemistry of gold nanorods is allowing the linking of various bifunctional groups like nucleic acid through strong Au-S bonding. Therefore the Adhesiveness of the composite film on the electrode surface can be improved by using Au nanorods [7]. Graphene/Au nanorods nanocomposite has been synthesized and used for fabricating NADH and ethanol biosensor [8]. The sensitive and stable enzyme-free biosensor with Au nanorods has been constructed [9]. An ultrasensitive graphene/Au nanorod/polythionine nanocomposite based electrochemical DNA biosensor was fabricated for human papillomavirus detection [10].

Metal-free Graphitic carbon nitride (g-C_3N_4) which is a polymer composed of carbon, nitrogen and some minor hydrogen amount has attracted great consideration due to its cheap cost, high thermal and chemical stability. Graphitic carbon nitride has applications in various fields such as photocatalytic splitting of water, photodegradation of organic pollutants and energy storage. Also, it is a good candidate to fabricate the electrochemiluminescence sensor because of its effective, strong and stable electrochemiluminescence emission. g-C_3N_4 in recent years has been applied for fabricating biosensor [11,12].

Electrochemical biosensors have gotten much attention because of convenient operation, rapid detection, good sensitivity and low cost [13]. They are appropriate for the development of a cheap and portable device for diagnoses of disease [14]. To fabricate DNA based electrochemical biosensor for detection of special DNA sequence; a probe which is a single strand nucleic acid is immobilized on the surface of solid support and coupled with the target that is specific single-strand DNA in the analyte. Upon the hybridization of the probe to target, redox labels make corresponding electrochemical signals [4, 15]. Redox labels can have two ways to interact with DNA: a) intercalative bind to the hybridized double-strand DNA, b) covalently tags to DNA strands [15].

Nanomaterials due to their unique advantages have offered modern approaches for low cost and sensitive detection of biomarkers. For preparing labels by loading signal tags such as dyes, metal ions, oligonucleotides, and quantum dots, various nanomaterials including gold nanoparticles, carbon nanotubes, magnetic have been used. Recently, titanium phosphate nanospheres functionalized with metal ion as the signal tag has been used in some electrochemical biosensors. Titanium phosphate can load the appropriate amount of metal ions and metal ions can be detected directly through square wave voltammetry (SWV) without treatment such as metal preconcentration [16-17].

Zap-70, tyrosine kinase protein is essential for T cell signaling but not found in normal b cells. A prognostic marker in identifying different forms of CLL is ZAP-70 in B-cells [4, 18]. Two major types of CLL with different survival times are distinguished with DNA analysis.

In this study, a sensitive electrochemical biosensor based on two DNAs as the target captures and TiP nanospheres functionalized with Pb^{2+} (TiP-Pb^{2+}) as a label was made for the detection of ZAP-70 DNA. The surface of GCE was modified by Au nanorods/g-C_3N_4 composite. The electrochemical signal was provided by Pb^{2+} ions which incorporated into the TiP nanospheres.

MATERIALS AND METHODS

Materials

Synthetic biotin-terminated DNA capture, capture DNA 1 and target sequences employed in this study (Table 1) were purchased from Bioneer Corporation (South Korea). All oligonucleotides were diluted with water and stored as a stock solution in a freezer. Phosphate buffer saline (PBS) with different pH values were prepared by mixing the stock solution of NaH_2PO_4 and Na_2HPO_4 .Then the pH was adjusted with NaOH (0.1 M) and H_3PO_4 (0.1 M). All solutions were prepared

with Milli-Q water (18MΩ cm resistivity) from a Millipore system. Melamine ($C_3H_6N_6$), 6-mercapto-1-hexanol (MCH), chloroauric acid ($HAuCl_4$), poly (allylamine hydrochloride), bovine serum albumin, docusate sodium, silver nitrate ($AgNO_3$), Sodium borohydride ($NaBH_4$) and all other reagents were prepared from Sigma-Aldrich.

Apparatus

X-ray diffraction patterns (XRD) of the sample were recorded at room temperature using CuKα radiation (D8Advance, BRUKER). The infrared spectrum was obtained on a FT-IR 6300 using KBr as the reference sample within a wavelength range of 400 - 4000cm^{-1}. The morphology and nanostructure of the sample were observed by field emission scanning electron microscopy (FESEM) of the TESCAN (MIRA3) which equipped with an energy dispersive X-ray spectrometer (EDS).

All electrochemical measurements were performed on a μAutolab III (Eco Chemie B.V.) potentiostat/galvanostat by NOVA 1.8 software. The utilized three-electrode system contained a reference electrode (saturated calomel electrode), an auxiliary electrode (platinum wire) and the working electrode (modified glassy carbon electrode with d=4 mm). All the measured potentials were reported with regards to the saturated calomel electrode.

Synthesis of g-C_3N_4 nanosheets

The bulk g-C_3N_4 was synthesized by polymerization of melamine molecules at high temperature. Melamine was heated under air condition at 600°C for 2 hours with a ramp rate of about 5°C/ min and stay 2h at 600°C for the heating process. The obtained product was yellow. g-C_3N_4 nanosheets were obtained by exfoliation of as–prepared bulk g-C_3N_4 in water. 0.4 g of bulk g-C_3N_4 power was dispersed in 800 ml of water and the mixture was ultrasound for 16 h. The unexfoliated g-C_3N_4 was removed by centrifugation [19, 20].

Synthesis of Au nanorods

Seed-mediated growth methods were used for the synthesis of gold nanorods [21].

Seed solution

CTAB solution (5 ml, 0.2M) was mixed with 5 ml of 0.0005 $HAuCl_4$, 0.6 ml of cold 0.01 M $NaBH_4$ was added to the stirred solution which resulted in the formation of a brownish-yellow solution. The seed solution was stirred a few minutes and then kept at 25°C[21].

Growth solution

5ml CTAB (0.2M) was added to 0.2 ml of 0.004 M $Ag(NO_3)$ solution at 25°C. Then 5 ml of 0.001 M $HAuCl_4$. After mixing the solution, 70μl of 0.0788 M ascorbic acid as a reducing agent changes the color of the solution from yellow to colorless.

In the end, 12μl of the seed solution was added to the growth solution at 27-30°C. The temperature of the medium was kept at 27-30° C [21].

Synthesis of Pb^{2+} functionalized TiP nanospheres

For synthesis TiP nanoparticles, 5g of docusate sodium salt (AOT) was dissolved into 32ml of ethanol and to get a turbid solution, H_3PO_4 (6 ml) was added. A mixture of 1.75g tetrabutyl titanate with 32 ml ethanol was dropped quickly. The mixture was stirred at 80° C for 6 h. To remove the residual phosphoric acid and surfactant, the product was washed with ethanol and water several times. Then 1 ml of (40$mg.ml^{-1}$) the colloid mixture of TiP nanospheres was dispersed into 30 ml of $Pb(NO_3)_2$ aqueous solution (10 Mm) and stirred at 50° C for 1 day. The hybrid nanospheres were obtained by centrifugation and rinsed with water several times. A mixture of TiP-pb^{2+} in water (20 $mg.ml^{-1}$) was prepared. Next, 2 ml of TiP-pb^{2+} hybrid was interspersed into 2 ml of polyallylamine hydrochloride aqueous solution and sonicated for 20 min. after this stage, the hybrid was washed with deionized water and dispersed into 2 ml of glutaraldehyde and sonicated for 5 min. then, the hybrid was washed with DI and PBS three times. 600μL of streptavidin protein solution (0.01 $mg.mL^{-1}$) was added into the product. This dispersion was shaken for 6h. the product was separated by centrifugation and was rinsed with PBS several times and suspended in 8 mL of tris buffer.

Modification of GCE with Au nanorods/g-C_3N_4 composite

Firstly, to obtain a mirror-like surface of electrode, the bare GCE was completely polished with a alumina–water slurry on a smooth polishing cloth. To remove the alumina residual particles, GCE was sonicated for 5 min in distilled deionized water and dried under nitrogen gas. The mixtures of g-C_3N_4 (1.0 mg/mL in H2O) and the GNR (100 mg/mL in H2O) were prepared separately. Before use,

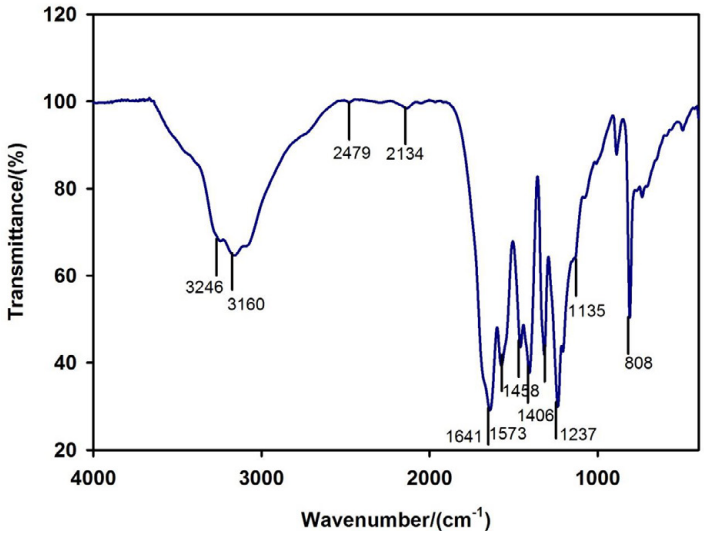

Fig.1. FTIR spectrum of g-C_3N_4

this mixture was exposed to mild ultra-sonication for 5 min. Thereafter 4.0 µL of the g-c4n3 solution was applied on the surface of the bare GCE and kept in isolating container until completely dried. Then, 4.0 µL of the GNR solution was also applied at the surface of the g-C_3N_4 -modified GCE and kept isolated in a high-humidity container. After stabilization of GNR on the surface of g-C_3N_4 sheets, 5 µL of buffer solution containing capture DNA1 (100 nM) applied to the modified GCE surface and incubated in a high-humidity container for 2 h at room temperature. SH group of capture DNA1 attached to the GNR surface. Then the electrode was washed with a washing solution and was incubated in the 6-mercapto-1-hexanol solution for 5 min. This electrode was washed with distilled water to remove the loose adsorbed materials.

DNA hybridization assay and measurement procedure

For accomplishment DNA hybridization assay, the GNR/ g-C_3N_4 composite modified electrode was incubated with 12 µL of mixture containing 5 µl of target DNA (different concentration or control samples), 5 µL of capture DNA2 (10Mm), 1µL of T4DNA ligase (1000 $U\mu L^{-1}$) and 1 µL of TBS for 4h at 37 ° C to complete the reaction of hybridization. In the following, the electrode was washed with TBS three times. Next modified GCE was incubated with 10 µL TiP–Pb^{2+}– streptavidin solution at 37 °C. To remove nonspecifically bound conjugates, the electrode was rinsed with TBS containing %1 bovine serum albumin. The electrochemical measurement was performed in 3 mL of acetate buffer (pH 4.6, 0.2 M) [19].

RESULTS AND DISCUSSION

Characterization

The chemical functional groups on the surface of g-C_3N_4 nanosheets with the high surface area were investigated by FTIR spectrum. The FTIR spectrum of g-C_3N_4 is displayed in Fig.1. The broad absorption peak at 3100-3300 cm^{-1} can be attributed to two sources: the stretching modes of secondary and primary amines and intermolecular hydrogen bonding interactions between amine groups [22]. The bands at 1200-1600 cm^{-1} are characteristic of C-N-C stretching vibrations in aromatic carbon nitride heterocycles [23, 24]. The sharp absorption peak centered at 808 cm^{-1} was related to the out of plane bending vibrations of triazine cycles [25].

To characterize the crystalline structure of the TiP, The XRD pattern of TiP was obtained (Fig.2). All peaks from the X-ray diffraction pattern clearly match with rhombohedral $Ti_4P_6O_{23}$, Titanium phosphate, (JCPDS card 39-0004). Observed peaks at 20.6°, 24.15°, 29.2°, 32.4°, and 36.4° are corresponded to (104), (113), (024), (116) and (030) miller indices, respectively. The crystallite size of $Ti_4P_6O_{23}$ was calculated from Scherrer`s equation(eq.1) and was found about 41.22 nm.

$$D = \frac{0.9\lambda}{\beta \cos\theta} \quad (1)$$

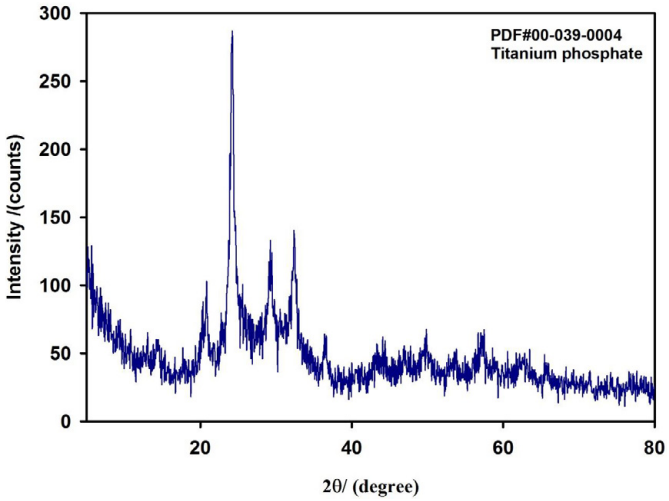

Fig.2. XRD pattern of TiP

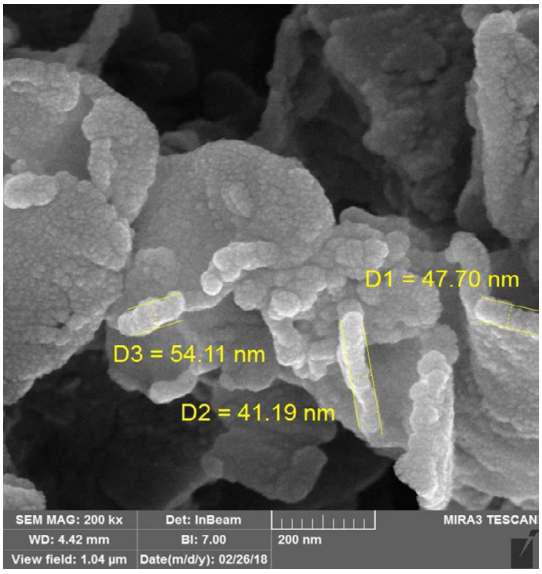

Fig.3. FESEM of Au nanorods /g-C_3N_4 nanocomposite

Where D is crystallite size, λ is the wavelength of Co kα radiation, β is the full width of half maximum of main intensity peak and θ is the brag`s angle.

Field emission Scanning Electron Microscope (SEM) image was prepared from Au nanorods /g-C_3N_4 nanocomposite (Fig.3). SEM image confirms the nanoscale dimensions of this composite. The elemental composition of Au nanorods / g-C_3N_4 composite was analyzed with EDS as depicted in Fig.4. The peaks of C, N, and Au were observed in the EDS spectrum. The presence of Au peaks in the EDS spectrum confirmed the formation of composite from Au nanorods and g-C_3N_4 sheets.

After the synthesis of hybrid pb^{2+} functionalized TiP nanospheres, polyallylamine hydrochloride was added and covalently bound to phosphate groups of TiP nanospheres and amine groups of polyallylamine hydrochloride. Then glutaraldehyde was used as crosslinking agents to attach [1]streptavidin protein on the surface of the TiP nanosphere.

Sensor performance

Au nanorods /g-C_3N_4 composite was immobilized on a surface of the glassy carbon electrode (GCE). Then S terminated DNA capture1 was attached to Au nanorods /g-C_3N_4 with Au-S bonds. DNA capture1 and DNA capture 2 own 12 and 11 bases, respectively that are complementary to the target DNA. If the target exists in the sample, the sandwich structure is formed. Then streptavidin on the surface of TiP-Pb^{2+} hybrids is attached to biotin which connected to DNA capture 2. 6-mercapto-1-hexanol was used to block nonspecific sites on the surface of electrode. Fig.5 indicates the schematic of the modified electrode and sandwich structure of analyte and probes.

The quantification of target DNA is performed by measurement the electrochemical current responses of Pb^{2+}. To electrochemical detection of target DNA, Square Wave Voltammetry (SWV) is an effective and sensitive method. Voltage scanning was done from -0.7 to -0.1 V with pulse amplitude 25 mV, pulse frequency 15 Hz, and quiet time 2 s. The electrochemical responses were recorded at −0.41 V for the quantitative valuation of target DNA. Different

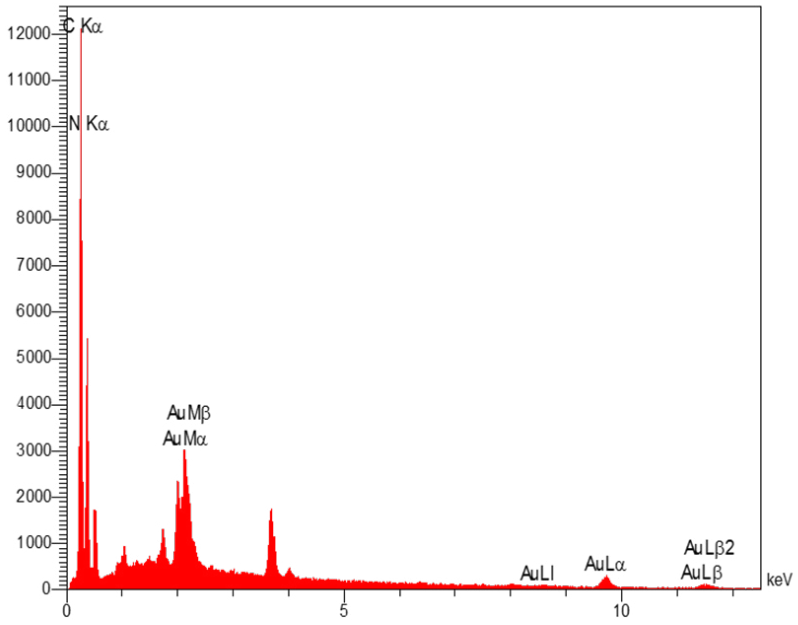

Fig.4. EDS spectrum of Au nanorods /g-C_3N_4 nanocomposite

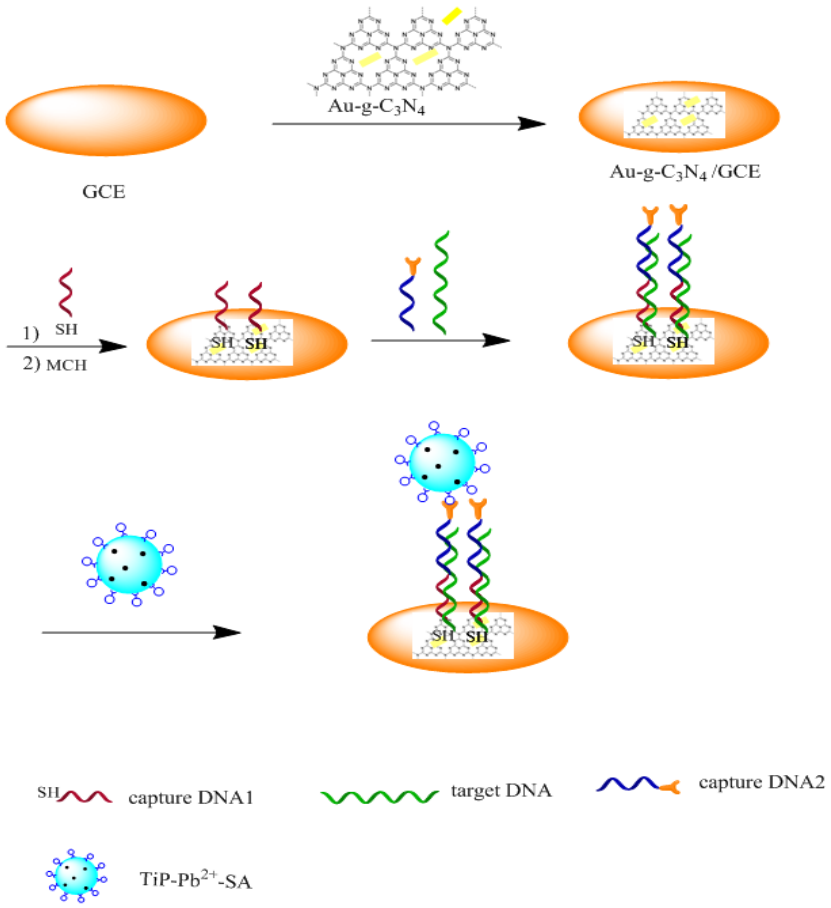

Fig.5. Schematic illustration of a modification of GCE

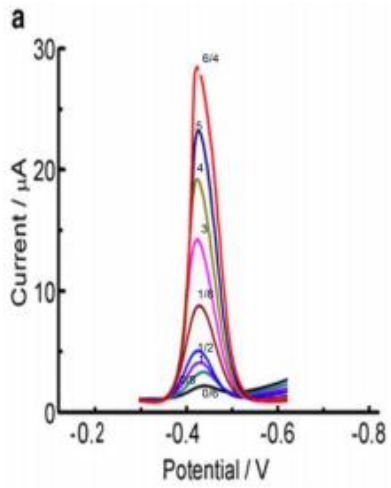

Fig.6. SWV of the modified electrode with different concentration of target DNA

concentrations of target DNA (6.4, 5, 4, 3, 1.8, 1.2, 1, 0.8, 0.6 nM) were measured to obtain a relation between the concentration of target DNA and electrochemical signal (current). Fig .6 depicts the SWV of the modified electrode with increasing the concentration of target DNA.

The amount of current from SWV revealed proper linear correlation versus target DNA concentrations in the range from 0.6 nM to 6.4 nM and with a detection limit of 20 pM. The linear equation of the most fitted straight line of current vs concentration of target DNA was obtained by Microsoft office Excel. The obtained equation was y=4.6489x-0.2325, which y is the current (μA) and x is the concentration of target DNA. The regression correlation coefficient (R^2) was calculated for this line about 0.9956.

The precision of the method was assessed by the evaluation of samples for five replicate measurements. The RSDs% (relative standard deviations) were 3.2, 1.6 and 1.6 for 10, 20, and 30nM target DNA, respectively.

CONCLUSION

Fabrication of new biosensor for accurate detection of CLL in the early stages is quite useful. Zap-70 DNA as a prognostic factor in CLL has been confirmed. The surface of GCE was modified with Au nanorods/g-C3N4 composite. Then two complementary strands of Zap-70 DNA as a probe and Pb^{2+}modified TiP nanoparticles as a signal unit were used for fabricating this biosensor. There was a linear relationship between the concentration of Zap-70 DNA and the SWV peak currents in the from 0.6 nM to 6.4 nM .The detection limit of 20 pM was obtained. This biosensor is a selective and sensitive biosensor for the detection of Zap-70 DNA as a biomarker of CLL.

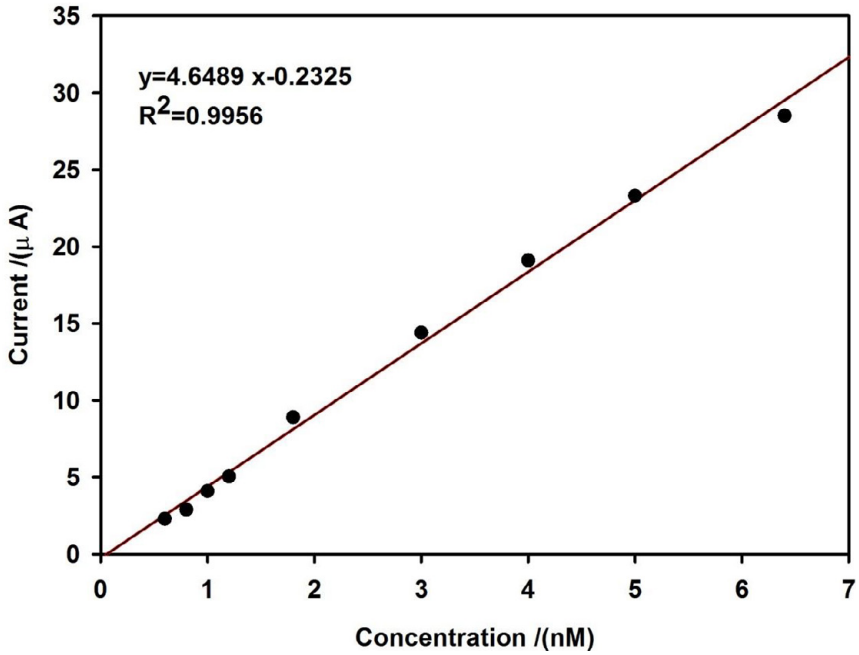

Fig.7. calibration curve of Aunanorods/g-C_3N_4 modified GCE electrode in different concentration of target DNA

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CONFLICT OF INTEREST

The authors declare no conflict of interest in this study.

REFERENCES

1. Zhang K, Ma H, Zhang L, Zhang Y. Fabrication of a Sensitive Impedance Biosensor of DNA Hybridization Based on Gold Nanoparticles Modified Gold Electrode. Electroanalysis. 2008;20(19):2127-33.
2. Shanafelt TD, Byrd JC, Call TG, Zent CS, Kay NE. Narrative Review: Initial Management of Newly Diagnosed, Early-Stage Chronic Lymphocytic Leukemia. Annals of Internal Medicine. 2006;145(6):435.
3. Choi Y-E, Kwak J-W, Park JW. Nanotechnology for Early Cancer Detection. Sensors. 2010;10(1):428-55.
4. Ensafi AA, Amini M, Rezaei B, Talebi M. A novel diagnostic biosensor for distinguishing immunoglobulin mutated and unmutated types of chronic lymphocytic leukemia. Biosensors and Bioelectronics. 2016;77:409-15.
5. Chu L-Q, Knoll W, Förch R. Plasma polymerized epoxide functional surfaces for DNA probe immobilization. Biosensors and Bioelectronics. 2008;24(1):118-22.
6. Su X, Robelek R, Wu Y, Wang G, Knoll W. Detection of Point Mutation and Insertion Mutations in DNA Using a Quartz Crystal Microbalance and MutS, a Mismatch Binding Protein. Analytical Chemistry. 2004;76(2):489-94.
7. Yan S, Xu X, Sheng Y, Sun H, Wu J, Tang L. Disposable Biosensor Based on Au Nanoparticles-Modified CdS Nanorod Arrays for Detection Cytochrome c. Journal of Nanoscience and Nanotechnology. 2011;11(12):10320-3.
8. Li L, Lu H, Deng L. A sensitive NADH and ethanol biosensor based on graphene–Au nanorods nanocomposites. Talanta. 2013;113:1-6.
9. N, Won Y-H, Huh K, Stanciu LA. Purdue e-Pubs Au nanospheres and nanorods for enzyme-free electrochemical biosensor applications Au nanospheres and nanorods for enzyme-free electrochemical biosensor applications. Biosens Bioelectron [Internet]. 2011 [cited 2020 Jan 7];26:4514–9. Available from: http://dx.doi.org/10.1016/j.bios.2011.05.012
10. Huang H, Bai W, Dong C, Guo R, Liu Z. An ultrasensitive electrochemical DNA biosensor based on graphene/Au nanorod/polythionine for human papillomavirus DNA detection. Biosensors and Bioelectronics. 2015;68:442-6.
11. Feng Q-M, Shen Y-Z, Li M-X, Zhang Z-L, Zhao W, Xu J-J, et al. Dual-Wavelength Electrochemiluminescence Ratiometry Based on Resonance Energy Transfer between Au Nanoparticles Functionalized g-C3N4 Nanosheet and Ru(bpy)32+ for microRNA Detection. Analytical Chemistry. 2015;88(1):937-44.
12. Liu Y, Ma H, Zhang Y, Pang X, Fan D, Wu D, et al. Visible light photoelectrochemical aptasensor for adenosine detection based on CdS/PPy/g-C3N4 nanocomposites. Biosensors and Bioelectronics. 2016;86:439-45.
13. Abi A, Lin M, Pei H, Fan C, Ferapontova EE, Zuo X. Electrochemical Switching with 3D DNA Tetrahedral Nanostructures Self-Assembled at Gold Electrodes. ACS Applied Materials & Interfaces. 2014;6(11):8928-31.
14. Alfonta L, Katz E, Willner I. Sensing of Acetylcholine by a Tricomponent-Enzyme Layered Electrode Using Faradaic Impedance Spectroscopy, Cyclic Voltammetry, and Microgravimetric Quartz Crystal Microbalance Transduction Methods. Analytical Chemistry. 2000;72(5):927-35.
15. Ensafi AA, Taei M, Rahmani HR, Khayamian T. Sensitive DNA impedance biosensor for detection of cancer, chronic lymphocytic leukemia, based on gold nanoparticles/gold modified electrode. Electrochimica Acta. 2011;56(24):8176-83.
16. Feng L-N, Bian Z-P, Peng J, Jiang F, Yang G-H, Zhu Y-D, et al. Ultrasensitive Multianalyte Electrochemical Immunoassay Based on Metal Ion Functionalized Titanium Phosphate Nanospheres. Analytical Chemistry. 2012;84(18):7810-5.
17. Cheng N, Tian J, Liu Q, Ge C, Qusti AH, Asiri AM, et al. Au-Nanoparticle-Loaded Graphitic Carbon Nitride Nanosheets: Green Photocatalytic Synthesis and Application toward the Degradation of Organic Pollutants. ACS Applied Materials & Interfaces. 2013;5(15):6815-9.
18. Moussay E, Wang K, Cho JH, van Moer K, Pierson S, Paggetti J, et al. MicroRNA as biomarkers and regulators in B-cell chronic lymphocytic leukemia. Proceedings of the National Academy of Sciences. 2011;108(16):6573-8.
19. Cheng F-F, He T-T, Miao H-T, Shi J-J, Jiang L-P, Zhu J-J. Electron Transfer Mediated Electrochemical Biosensor for MicroRNAs Detection Based on Metal Ion Functionalized Titanium Phosphate Nanospheres at Attomole Level. ACS Applied Materials & Interfaces. 2015;7(4):2979-85.
20. Zhang X, Xie X, Wang H, Zhang J, Pan B, Xie Y. Enhanced Photoresponsive Ultrathin Graphitic-Phase C3N4 Nanosheets for Bioimaging. Journal of the American Chemical Society. 2012;135(1):18-21.
21. Nikoobakht B, El-Sayed MA. Preparation and Growth Mechanism of Gold Nanorods (NRs) Using Seed-Mediated Growth Method. Chemistry of Materials. 2003;15(10):1957-62.
22. Lotsch BV, Schnick W. From Triazines to Heptazines: Novel Nonmetal Tricyanomelaminates as Precursors for Graphitic Carbon Nitride Materials. Chemistry of Materials. 2006;18(7):1891-900.
23. Lee S, Yu J-S, Hwang S. Template-directed synthesis of highly ordered nanoporous graphitic carbon nitride through polymerization of cyanamide Material modification View project Particle size determination View project Template-directed synthesis of highly ordered nanoporous graphitic carbon nitride through polymerization of cyanamide. 2006 [cited 2020 Jan 7];

Available from: www.elsevier.com/locate/apsusc
24. Sun L, Zhao X, Jia C-J, Zhou Y, Cheng X, Li P, et al. Enhanced visible-light photocatalytic activity of g-C3N4–ZnWO4 by fabricating a heterojunction: investigation based on experimental and theoretical studies. Journal of Materials Chemistry. 2012;22(44):23428.
25. Chen Y, Li J, Hong Z, Shen B, Lin B, Gao B. Origin of the enhanced visible-light photocatalytic activity of CNT modified g-C3N4 for H2 production. Physical Chemistry Chemical Physics. 2014;16(17):8106.