Journal of Biology and Today's World

Journal home page: http://journals.lexispublisher.com/jbtw

Received: 13 April 2016 • Accepted: 20 August 2016

Research

doi:10.15412/J.JBTW.01050801

Electrochemical Genosensing of *Leishmania major* using Gold Hierarchical Nanoleaflets

Marzieh Moradi[1], Naghmeh Sattarahmady[1,2*], Gholamreza Hatam[3,4], Hossein Heli[1♠]

[1] Nanomedicine and Nanobiology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

[2] Department of Medical Physics, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

[3] Basic Sciences in Infectious Diseases Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

[4] Department of Parasitology and Mycology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

*Correspondence should be addressed to Naghmeh Sattarahmady, Nanomedicine and Nanobiology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran; Department of Medical Physics, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran Tel: +987132349332; Fax: +987132349332; Email: sattarahmady@yahoo.com; nsattar@sums.ac.ir.

♠Co-Correspondence should be addressed to Hossein Heli, Nanomedicine and Nanobiology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran; Tel: +987132349332; Fax: +987132349332; Email: hheli7@yahoo.com; heli@sums.ac.ir.

ABSTRACT

A label- and PCR-free electrochemical genosensor was fabricated for identification of *Leishmania* parasites. Gold hierarchical nanoleaflets were synthesized by electrodeposition in the presence of spermidine in the deposition solution. A *Leishmania major* specific single stranded DNA probe was immobilized on the surface of nanoleaflets and ferrocyanide was employed as a hybridization marker. Hybridization of the immobilized probe with targets was investigated under the selected conditions. The genosensor could detect a synthetic DNA target in a range of 1.0×10^{-12} to 1.0×10^{-20} mol L^{-1} with a limit of detection of 2.98×10^{-21} mol L^{-1}, and genomic DNA in a range of 0.5 to 15 ng μL^{-1} with a limit of detection of 0.11 ng μL^{-1}. The genosensor was successfully applied for detection of *Leishmania major* in patient samples.

Key words: Leishmaniasis, Genosensor, DNA biosensor, Gold, Nanoleaflets.

Journal of Biology and Today's World is published by *Lexis Publisher*; Journal p-ISSN 2476-5376; Journal e-ISSN 2322-3308.

1. INTRODUCTION

Leishmaniasis is a geographically widespread severe disease with an increasing incidence of two million cases per year and 350 million people from 88 countries are at risk (1). *Leishmania major* is one of the many protozoan parasites of the genus *Leishmania* that causes lishmaniasis (2). These pathogens exist in two distinct parasitic stages, namely, in promastigote and amastigote stages. Promastigote is the infective-stage and transmitted by the bite of a female phlebotomine sandflies belonging to 30 different species throughout the world (3). There are methods for the diagnosis of leishmaniasis including:

a) Observation of the parasite in the stained specimen of tissues, in vitro culture or animal inoculation by light microscopy;

b) Parasite DNA detection in tissue samples, and

c) Immune analysis of parasite antigen in tissues, blood or urine samples by detection of antileishmanial antibodies (immunoglobulin), or by assay for *Leishmania*-specific cell-mediated immunity (4). A definitive diagnosis is identification of amastigotes in a Wright-Giemsa-stained touch preparation or through isolation of the parasites in culture (5). The sensitivity of the tissue examination, except in the case of splenic aspirate, is low. Identification of amastigotes requires considerable expertise and training, and is subject to the ability of the observer (6). In the past decade, polymerase chain reaction (PCR)-based techniques have been used more in diagnosis of leishmaniasis, however, the demonstration of parasites with PCR requires a well-established laboratory and equipment such as a thermal cycler and a system to detect and analyze amplicons (7). Wide-scale genetic testing requires development of easy-to-use, fast, inexpensive, miniaturized analytical devices. Electrochemical biosensors attract considerable interest due to their tremendous promise in obtaining sequence-specific information in a faster, simpler, and cheaper manner

compared to traditional assays (8). Also, in order to excellent economic prospects, such the devices offer innovative routes for interfacing (at the molecular level) the DNA-recognition and signal-transduction elements (9). Researches in this field would be beneficial to recognize the biotic origin, inheritance, growth, and evolution and create new methods for diagnosis, treatment, and prevention of human diseases (10, 11). In the present study, hierarchical nanoleaflets were electrodeposited using spermidine as a shape-directing agent. A specific single-stranded DNA (ssDNA) probe for *Leishmania major* immobilized on the nanoleaflets surface to fabricate a signal-on and PCR- and label-free electrochemical genosensor; this genosensor was applied to detection of *Leishmania major* in patient samples successfully.

2. MATERIALS AND METHODS

2.1. Materials

All chemicals were prepared with analytical grade and purchased from Sigma (USA) and Scharlau (Spain) that used without further purification. All solutions were prepared by redistilled water. A ssDNA sequence (probe oligonucleotide, pDNA) was designed based on the genomic sequence of *Leishmania major*. pDNA and a complementary-sequence oligonucleotide (target oligonucleotide, tDNA) were purchased from Takapouzist Co. (Iran). The oligonucleotide sequences were as follows**:**
- pDNA sequence:

5'-SH-$(CH_2)_6$-TTTTTTTTTT-AACCCACTAAAGCGTCACCCAACA-3'
- tDNA sequence:
5'-TGTTGGGTGACGCTTTAGTGGGTT-3'

The stock solutions of oligonucleotides were prepared with 20 mmol L^{-1} Tris-HCl buffer, pH 7.4 solution (Tris) and kept frozen.

2.2. Synthesis of gold nanoleaflets

Gold nanoleaflets were synthesized on a polycrystalline gold disk (Au, 2 mm of diameter) surface by electrodeposition method. A gold disk electrode was firstly polished on a polishing pad by 0.05 µm alumina powder lubricated by water to attain a mirror-like surface. The electrode was further electropolished by immersion in a 0.5 mol L^{-1} H_2SO_4 solution and applying 20 consecutive cyclic potential in the range of cathodic to anodic edges of the electrolyte stability. Upon this pretreatment, a clean and stable gold surface was obtained. The electrode was then placed in a solution of 0.5 mol L^{-1} H_2SO_4 + 0.02 mol L^{-1} $HAuCl_4$ + 0.15 mol L^{-1} spermidine. Electrodeposition was performed at 0.0 V (vs. Ag/AgCl, 3 mol L^{-1} KCl) for 300 s. The resultant electrode was the gold disk electrode covered by gold nanoleaflets (Au-AuNL). Au-AuNL electrode was then rinsed thoroughly with ethanol and distilled water and dried at room temperature.

2.3. Characterization of gold nanoleaflets

Field emission scanning electron microscopy (FESEM) images were recorded by a Zeiss, Sigma-IGMA/VP (Germany) equipped with energy dispersive X-ray spectroscopy (EDS) capability.

2.4. Electrochemical studies

Electrochemical studies were performed in a conventional three-electrode cell powered by a µ-Autolab potentiostat/galvanostat that runs by a computer through GPES 4.9 software. An Ag/AgCl, 3 mol L^{-1} KCl, and a platinum rod were used as the reference and counter electrodes respectively. The real surface areas of the Au and Au-AuNL electrodes were measured electrochemically. The electrodes were transferred to a solution of 0.5 mol L^{-1} KCl + 0.5 mmol L^{-1} $K_4[Fe(CN)_6]$ and cyclic voltammograms at different potential sweep rates were recorded. Using the Randles-Sevcik equation (12) and the value of 7.60×10^{-6} cm s^{-1} for the diffusion coefficient of $[Fe(CN)_6]^{4-}$ (13), the real surface areas of the electrodes were obtained. Detection of DNA hybridization was performed electrochemically by differential pulse voltammetry (DPV) in a 10 mL solution of Tris and recording the oxidation peak of $[Fe(CN)_6]^{4-}$. For DPV, a pulse width of 25 mV, a pulse time of 50 ms, and a scan rate of 10 mV s^{-1} were employed. The concentrations of tDNA solutions were quantified as the $[Fe(CN)_6]^{4-}$ oxidation peak currents.

2.5. Immobilization of pDNA

pDNA was immobilized on gold nanoleaflets by dropping 10 µL of 10 µmol L^{-1} pDNA solution on the Au-AuNL electrode surface and remained intact at 4 ºC for 8 h [14]. The electrode was then rinsed thoroughly with Tris to remove unabsorbed pDNA. Afterward, the electrode was further treated with 10 µL 6-mercapto-1-hexanol (1.0 mmol L^{-1}) for 30 min. The resultant electrode was denoted as Au-AuNL-pDNA (the genosensor).

2.6. Hybridization with tDNA

The hybridization process was performed by dropping 10 µL of Tris containing various concentrations of tDNA on the genosensor surfaces for 60 min at 37 °C (14). During this procedure, a small vial was tightly fit on the electrodes' body to prevent quick evaporation of the droplet. Then, the electrodes were rinsed with Tris to remove the un-hybridized tDNA, and then it was transferred to a solution containing Tris + 0.5 mmol L^{-1} $[Fe(CN)_6]^{4-}$ and differential pulse voltammograms (DPVs) were recorded.

2.7. Parasite culture and genomic DNA extraction

The cryopreserved *Leishmania major* (MRHO/IR/75/ER) was received from Tehran University of Medical Sciences, Tehran, Iran. It was reactivated, and cultivated in RPMI 1640 medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum (Gibco) and 1% pen-strep (Sigma) at 24 °C. The promastigotes were counted daily in

a Neubauer chamber until the concentration of 1×10^7 mL^{-1} parasites. Approximately, 10^7 phase promastigotes of isolates were harvested from the culture by centrifugation. The pellets were washed 3-4 times with chilled 50 mmol L^{-1} phosphate buffer saline (pH 7.0 and 0.7% NaCl) and suspended in 750 mL of NET buffer (5 mol L^{-1} NaCl, 0.5 mol L^{-1} EDTA, 1 mol L^{-1} Tris buffer, pH 8.0). The cells were lysed with Proteinase K (100 mg mL^{-1}) and 1% sodium dodecyl sulphate for 3-4 h at 56 °C. Total DNA was extracted by the YTA Genomic DNA extraction mini kit (Yekta Tajhiz Azma, Iran) following the manufacturer's instructions, and DNA was eluted in 100-150 µL of elution buffer. The parasitic DNA concentration was determined by a Thermo Scientific NanoDrop 2000c (USA), and was then diluted.

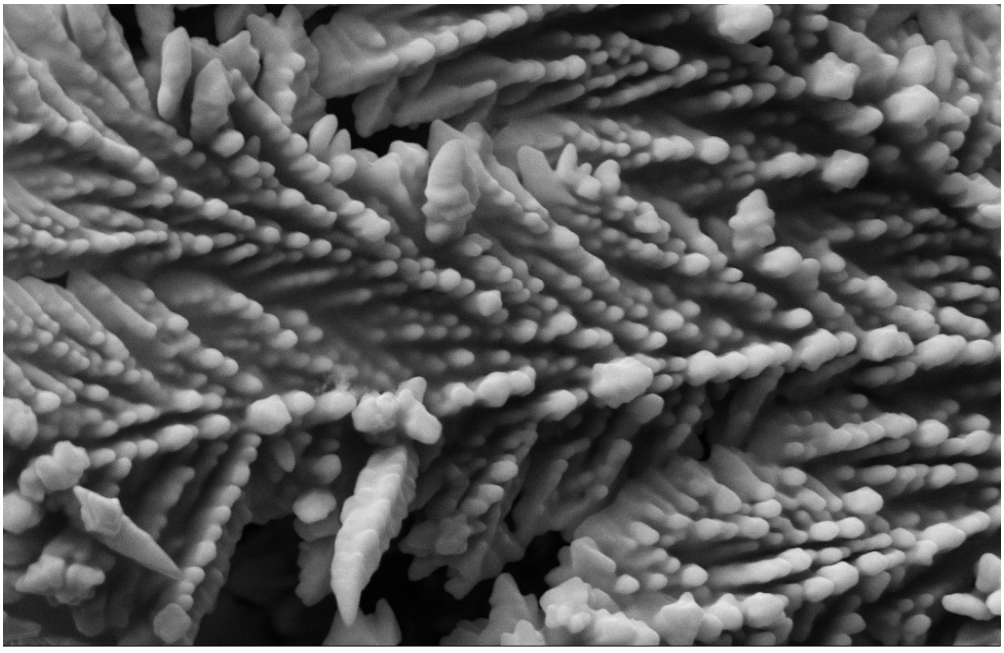
2.8. Genomic DNA extraction from human samples and PCR

Biopsy specimens of five human samples with cutaneous leishmaniasis were collected after sterilization of the lesion area on the slides, stained by Giemsa and microscopically characterized. Parasite DNA was extracted from 100 to 200 µL of the infected blood samples by the YTA Genomic DNA extraction Mini kit (Yekta Tajhiz Azma, Iran) following the manufacturer's instructions, and DNA was eluted in 100-150 µL of elution buffer. PCR, followed by gel electrophoreses, was performed to identify *Leishmania major* in the human samples with cutaneous leishmaniasis. PCR primers were based on the non-protein coding region (AB678349.1) of *Leishmania major* minicircle kDNA (Iranian Standard strain MCAN/IR/97/LON490, isolate: IranJWmaj) gene region (15), and purchased from Sinaclon, Iran. The YTA kit for tissue was employed also.

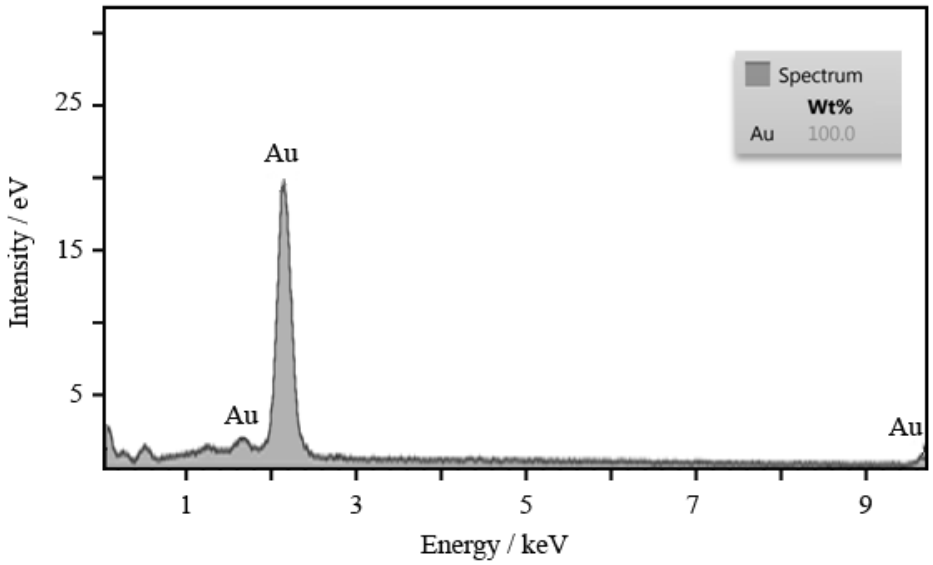
3. RESULTS AND DISCUSSION

Figure 1(a), (b) represents FESEM images of gold hierarchical nanoleaflets at different magnifications. The gold sample resembles tree leaflet with midribs and laminas at low magnification, and includes arrays of nanoparticles of 40-100 nm at high magnification. Figure 1(c) shows EDS of the nanoleaflets confirming the purity of the sample. Gold nanostructures provide suitable substrates to immobilize biorecognition elements and facilitate the hybridization efficiency, as also reported previously (16-18). The real surface area of the gold nanoleaflets was obtained as 0.66 cm^{-2}, and the geometric surface area was 0.0314 cm^{-2} representing a roughness factor of 21. This indicates that the Au-AuNL electrode had a large real surface area for immobilization of pDNA with a high surface concentration. During the electrodeposition process, gold atoms and clusters were rapidly electrodeposited on the Au electrode surface, following by promotion of gold nucleation (19). Simultaneously, spermidine is preferentially adsorbed on the (1 1 1) plane surface of the gold nuclei through a charge mechanism of donation/back-donation (20). Spermidine adsorption was performed by multi-amine functional group-gold interaction and provides a positively charged amine layer; It causes further electrostatic adsorption of $AuCl_4^-$ ions and preferential growth of gold along the (1 1 1) directions by the diffusion-limited aggregation mechanism (21).

(a)



(b)



(c)

Figure 1. FESEM images (a, b) and EDS (c) of the gold hierarchical nanoleaflets

Figure 2 (a) shows differential pulse voltammograms (DPVs) of the $[Fe(CN)_6]^{4-}$ solution recorded using designed genosensor, before and after hybridization with different concentrations of tDNA. The peak current of $[Fe(CN)_6]^{4-}$ using the genosensor before hybridization was the lowest, and upon hybridization of pDNA with tDNA, the peak current increased. $[Fe(CN)_6]^{4-}$ was repelled from the negative surface charge of genosensor (acquiring from the phosphate backbone of the pDNA structure), and upon hybridization, the net negative surface charge was reduced and $[Fe(CN)_6]^{4-}$ can approach to the surface. The dependency of the peak current on the tDNA concentration is shown in Figure 2 (b). The plot is linear with a regression equation of $y = (0.2632\pm0.0091)x + (6.6329\pm0.1483)$ in the range of 1.0×10^{-12} to 1.0×10^{-20} mol L^{-1} of tDNA. A limit of detection (LOD, equal to 3SD/m (22) which SD is the standard deviation of blank signal, and m is the slope of the calibration curve) was obtained as 2.98×10^{-21} mol L^{-1}. Gold hierarchical nanoleaflets provide a high surface area needing for preparation of a high surface concentration and dense layer for pDNA. On the other hand, gold nanostructures may increase the deflection of immobilized pDNA strands, alter their interactions, and cause appropriate orientations (23, 24). The results were more accessibility for tDNA (25) to hybridize with pDNA through an enhanced kinetics.

Finally, formation of a dense layer of pDNA on the surface can change the diffusion regime of $[Fe(CN)_6]^{4-}$ from linear to radial (26).

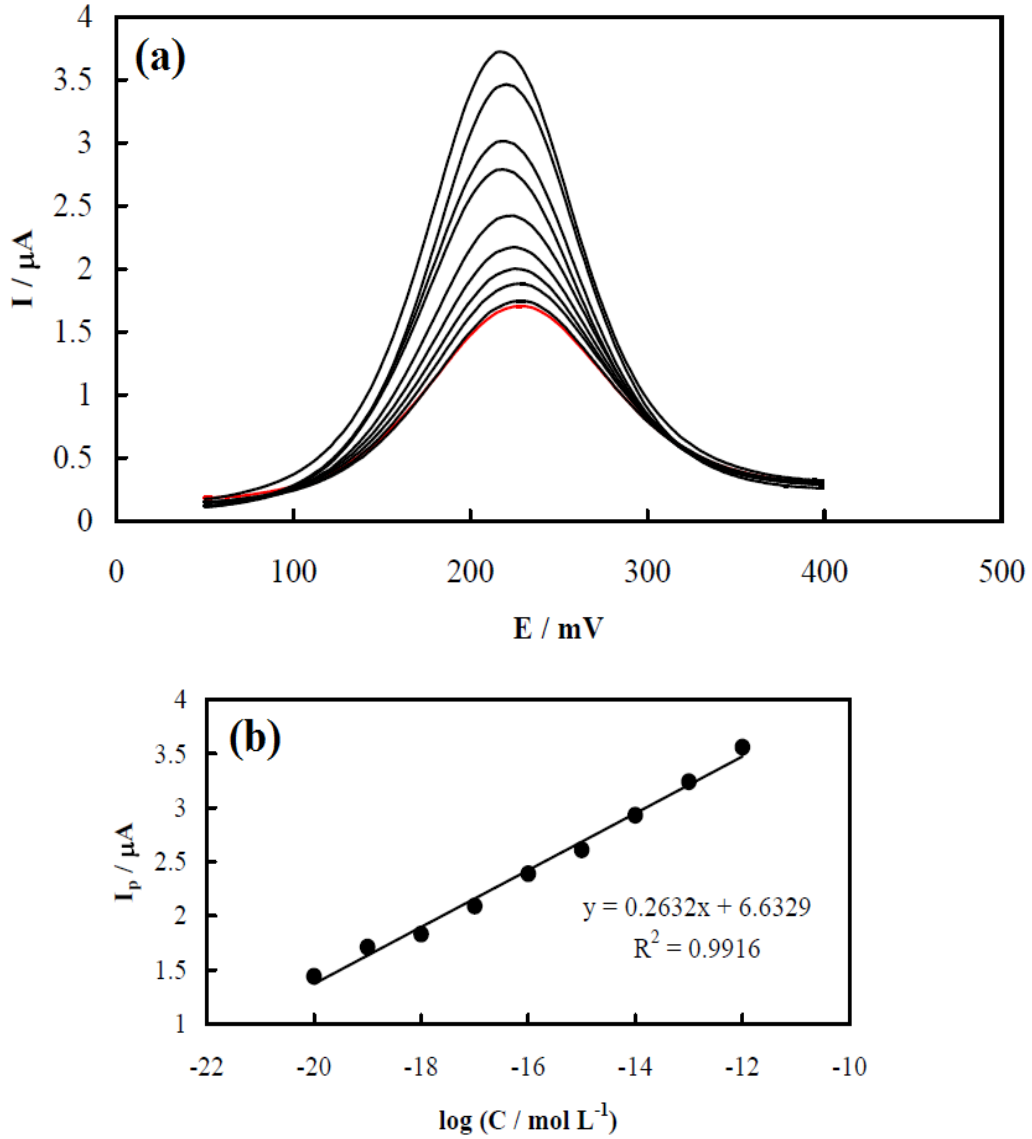


Figure 2. a) DPVs of $[Fe(CN)_6]^{4-}$ solution recorded using the genosensor, before and after hybridization with different concentrations of tDNA of 1.0×10-20, 1.0×10-19, 1.0×10-18, 1.0×10-17, 1.0×10-16, 1.0×10-15, 1.0×10-14, 1.0×10-13 and 1.0×10-12 mol.L^{-1}. The red curve is for the absence of tDNA. b) The related corresponding calibration curve

In order to detection of genomic DNA, the DNA genome was extracted from *Leishmania major* following by determination of DNA concentrations of the extracted samples. Afterwards, genome solutions with different concentrations were prepared. To de-hybridize the strands, all samples were placed in water at 90 °C for 10 min before analysis. Then, DPVs of $[Fe(CN)_6]^{4-}$ solution were recorded using the genosensor before and after hybridization with different concentrations of the genomic target; the data is presented in Figure 3 (a). The corresponding calibration curve for the data is also shown in Figure 3 (b). The *Leishmania major* genome was determined in a linear concentration range of 0.5 to 15 ng μL^{-1}. A LOD was obtained as 0.11 ng μL^{-1}. The LOD obtained here, is better than that reported previously (27).

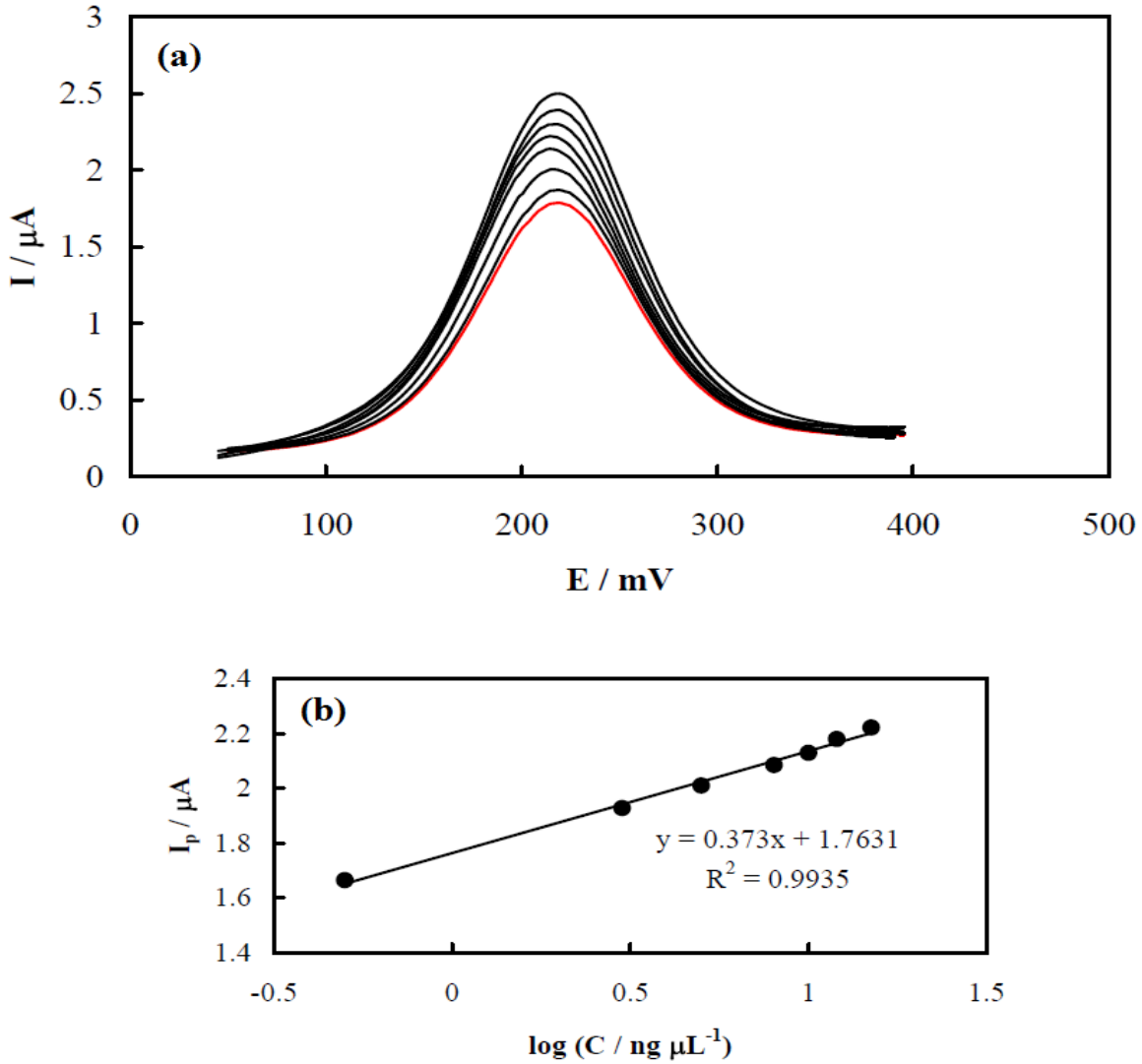


Figure 3. a) DPVs of $[Fe(CN)_6]^{4-}$ solution recorded using the genosensor, before and after hybridization with different concentrations of DNA genome of 0.5, 3, 5, 8, 10, 12 and 15 ng µL^{-1}. The red curve is for the absence of DNA genome. b) The corresponding calibration curve

To evaluate the regeneration of mentioned genosensor, it was hybridized with 1.0×10^{-15} mol L^{-1} tDNA firstly, and then placed in water at 90 °C for 5 min to de-hybridize the formed double stranded DNA. The genosensor was then re-hybridized with the same tDNA concentration (n=5), and DPVs were recorded each time separately; the related data is shown in Figure 4. Based on the results, a relative standard deviation (RSD) was obtained as 7.1% for the regeneration of this genosensor.

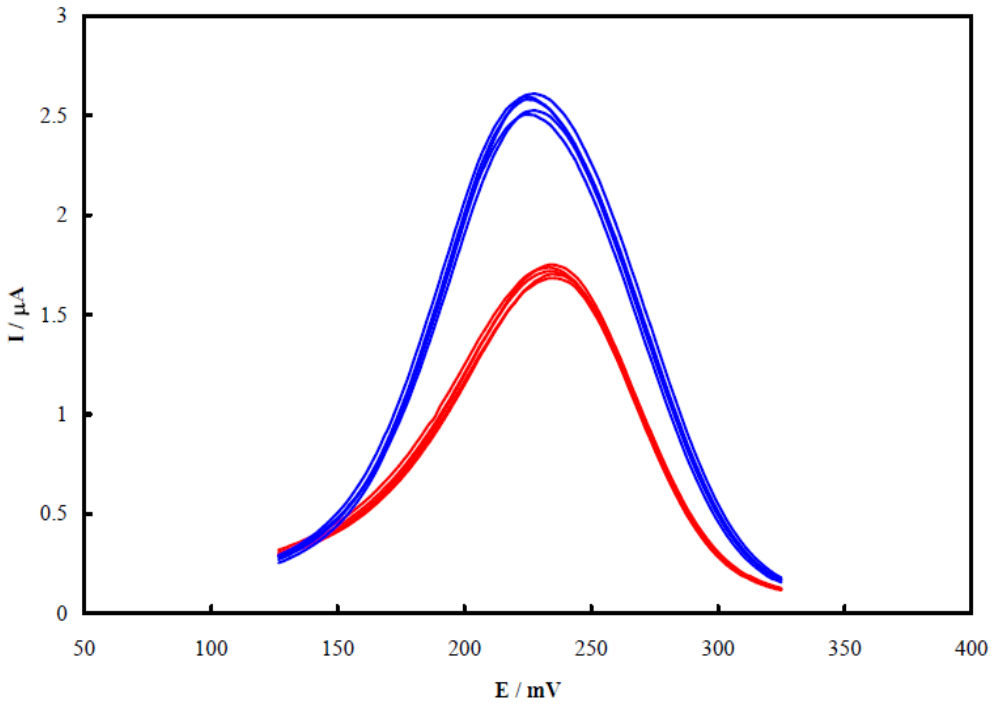


Figure 4. DPVs of $[Fe(CN)_6]^{4-}$ solution recorded using the genosensor before (red curves) and after re-hybridization (n=5, blue curves) with 1.0×10-15 mol L^{-1} tDNA

In order to evaluate the stability of the genosensor, DPVs in $[Fe(CN)_6]^{4-}$ solution using the genosensor for 1.0×10^{-15} mol L^{-1} tDNA over 15 days were recorded, while the genosensor was stored in Tris in the refrigerator at 4 °C and DPVs were recorded in the same conditions. The data are presented in Figure 5 (a), and the variation in the DPV peak currents over time is presented in Figure 5 (b). Based on the results, changes in the peak currents were irregular with a RSD value (6.5%).

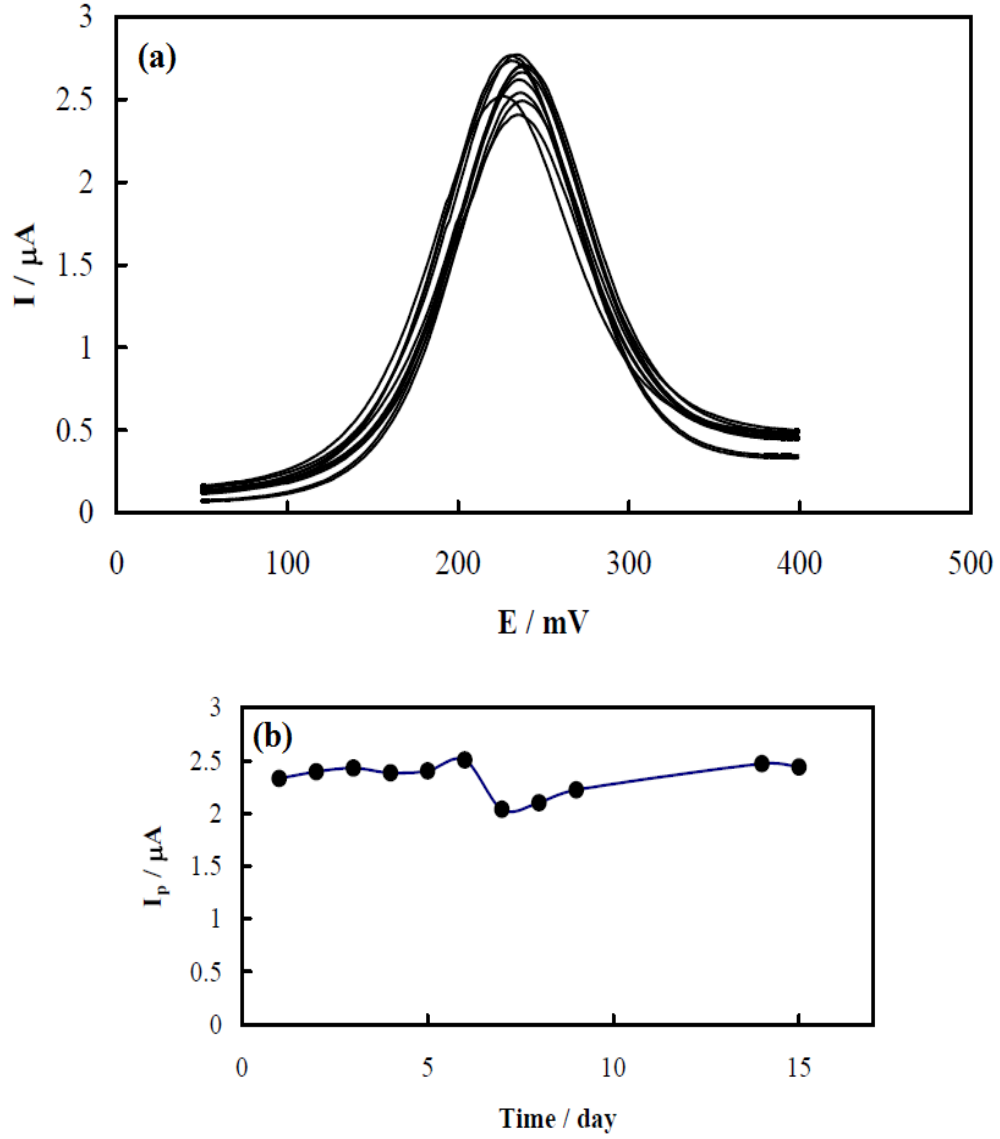


Figure 5. a) DPVs the $[Fe(CN)_6]^{4-}$ solution using the genosensor for 1.0×10^{-15} mol L^{-1} tDNA over 15 days. b) Variation in the DPV peak currents over time

In order to employ the genosensor for diagnosis of cutaneous leishmaniasis in patients, biopsy specimens of five human samples with leishmaniasis were collected. Then, the genomes from samples were extracted, and the presence of the *Leishmania major* was confirmed by PCR and gel electrophoresis. The concentration of DNA in the samples were then determined and a similar concentration of 5 ng μL^{-1} was prepared for all the samples. They were then de-hybridized and analyzed using the genosensor. Increment in the peak currents in DPVs was recorded for theses samples, and the value of the limit of quantitation of the genosensor (LOQ, 10SD/m (22), n=10) was measured. It was found that the increments in the peak currents for the samples containing *Leishmania major* were meaningfully higher than LOQ of the genosensor. Therefore, the genosensor has a capability of *Leishmania major* detection in the samples from patients also.

4. CONCLUSION

Electrodeposition was employed to preparation of gold hierarchical nanoleaflets. Electrodeposition parameters and the additive in the deposition (spermidine) had main roles in formation of the gold nanostructure. Change in these parameters may result in formation of the other nanostructures. The nanoleaflets had a high surface area and immobilization of the thiolated pDNA on this surface resulted in the fabrication of a high sensitive electrochemical genosensor. The genosensor is label-free, PCR-free and signal-on and applied to detect cutaneous leishmaniasis.

ACKNOWLEDGMENT

We would also like to thank the Research Council of Shiraz University of Medical Sciences (11944) and the Iran National Science Foundation (INSF) for supporting this research.

FUNDING/SUPPORT

Not mentioned any Funding/Support by authors.

AUTHORS CONTRIBUTION

Marzieh Moradi performed experiments, some data analyses, and prepared the first manuscript draft. Naghmeh Sattarahmady provided the main idea and some discussion about the results. Gholamreza Hatam provided some technical equipment, materials and biologicals, and some idea, Hossein Heli provided some ideas and data analyses, and prepared the figures and final version of the manuscript. All authors reviewed the manuscript and made discussion on the results.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this paper.

REFERENCES

1. Alvar J, Velez ID, Bern C, Herrero M, Desjeux P, Cano J, et al. Leishmaniasis worldwide and global estimates of its incidence. PloS one. 2012;7(5):e35671.
2. Andreadou M, Liandris E, Gazouli M, Taka S, Antoniou M, Theodoropoulos G, et al. A novel non-amplification assay for the detection of Leishmania spp. in clinical samples using gold nanoparticles. Journal of microbiological methods. 2014;96:56-61.
3. Bard AJ, Faulkner LR. Fundamentals and applications. Electrochemical Methods, 2nd ed; Wiley: New York. 2001.
4. Bin X, Sargent EH, Kelley SO. Nanostructuring of sensors determines the efficiency of biomolecular capture. Analytical chemistry. 2010;82(14):5928-31.
5. Chappuis F, Sundar S, Hailu A, Ghalib H, Rijal S, Peeling RW, et al. Visceral leishmaniasis: what are the needs for diagnosis, treatment and control? Nature reviews microbiology. 2007;5(11):873-82.
6. Desjeux P. Leishmaniasis: public health aspects and control. Clinics in dermatology. 1996;14(5):417-23.
7. Drummond TG, Hill MG, Barton JK. Electrochemical DNA sensors. Nature biotechnology. 2003;21(10):1192-9.
8. Gatti S, Gramegna M, Klersy C, Madama S, Bruno A, Maserati R, et al. Diagnosis of visceral leishmaniasis: the sensitivities and specificities of traditional methods and a nested PCR assay. Annals of Tropical Medicine & Parasitology. 2004;98(7):667-76.
9. Hill HD, Millstone JE, Banholzer MJ, Mirkin CA. The role radius of curvature plays in thiolated oligonucleotide loading on gold nanoparticles. ACS nano. 2009;3(2):418-24.
10. Hoft RC, Ford MJ, McDonagh AM, Cortie MB. Adsorption of amine compounds on the Au (111) surface: a density functional study. The Journal of Physical Chemistry C. 2007;111(37):13886-91.
11. Karadeniz H, Kuralay F, Abaci S, Erdem A. The recent electrochemical biosensor technologies for monitoring of nucleic acid hybridization. Current Analytical Chemistry. 2011;7(1):63-70.
12. Lapierre-Devlin MA, Asher CL, Taft BJ, Gasparac R, Roberts MA, Kelley SO. Amplified electrocatalysis at DNA-modified nanowires. Nano letters. 2005;5(6):1051-5.
13. Lord H, Kelley SO. Nanomaterials for ultrasensitive electrochemical nucleic acids biosensing. Journal of Materials Chemistry. 2009;19(20):3127-34.
14. Marsden PD, Jones TC, Chang K, Bray R. Clinical manifestations, diagnosis and treatment of leishmaniasis. Leishmaniasis(Human Parasitic Diseases Vol 1). 1985:183-98.
15. Miller JC, Miller JN. Statistics for analytical chemistry. 1988.
16. Moradi M, Sattarahmady N, Rahi A, Hatam G, Sorkhabadi SR, Heli H. A label-free, PCR-free and signal-on electrochemical DNA biosensor for Leishmania major based on gold nanoleaves. Talanta. 2016;161:48-53.
17. Pandey K, Pandey BD, Mallik AK, Acharya J, Kato K, Kaneko O, et al. A new molecular surveillance system for leishmaniasis. The American journal of tropical medicine and hygiene. 2014;90(6):1082-6.
18. Pividori M, Merkoci A, Alegret S. Electrochemical genosensor design: immobilisation of oligonucleotides onto transducer surfaces and detection methods. Biosensors and Bioelectronics. 2000;15(5):291-303.
19. Rahi A, Sattarahmady N, Heli H. Zepto-molar electrochemical detection of Brucella genome based on gold nanoribbons covered by gold nanoblooms. Scientific reports. 2015;5.
20. Rahi A, Sattarahmady N, Heli H. Label-free electrochemical aptasensing of the human prostate-specific antigen using gold nanospears. Talanta. 2016;156:218-24.
21. Reithinger R, Dujardin J-C. Molecular diagnosis of leishmaniasis: current status and future applications. Journal of Clinical Microbiology. 2007;45(1):21-5.
22. Rosi NL, Giljohann DA, Thaxton CS, Lytton-Jean AK, Han MS, Mirkin CA. Oligonucleotide-modified gold nanoparticles for intracellular gene regulation. Science. 2006;312(5776):1027-30.
23. Shaw JJ. Taxonomy of the genus Leishmania: present and future trends and their implications. Memórias do Instituto Oswaldo Cruz. 1994;89(3):471-8.
24. Soleymani L, Fang Z, Sargent EH, Kelley SO. Programming the detection limits of biosensors through controlled nanostructuring. Nature Nanotechnology. 2009;4(12):844-8.
25. Wang J, Duan G, Li Y, Liu G, Dai Z, Zhang H, et al. An invisible template method toward gold regular arrays of nanoflowers by electrodeposition. Langmuir. 2013;29(11):3512-7.
26. Wang S-F, Xu Q. Electrochemical parameters of ethamsylate at multi-walled carbon nanotube modified glassy carbon electrodes. Bioelectrochemistry. 2007;70(2):296-300.
27. Witten Jr T, Sander LM. Diffusion-limited aggregation, a kinetic critical phenomenon. Physical review letters. 1981;47(19):1400.