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An electrochemical genosensor for *Leishmania major* detection based on dual effect of immobilization and electrocatalysis of cobalt-zinc ferrite quantum dots

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ABSTRACT

Identification of *Leishmania* parasites is important in diagnosis and clinical studies of leishmaniasis. Although epidemiological and clinical methods are available, they are not sufficient for identification of causative agents of leishmaniasis. In the present study, quantum dots of magnetic cobalt-zinc ferrite ($Co_{0.5}Zn_{0.5}Fe_2O_4$) were synthesized and characterized by physicochemical methods. The quantum dots were then employed as an electrode modifier to immobilize a 24-mer specific single stranded DNA probe, and fabrication of a label-free, PCR-free and signal-on electrochemical genosensor for the detection of *Leishmania major*. Hybridization of the complementary single stranded DNA sequence with the probe under the selected conditions was explored using methylene blue as a redox marker, utilizing the electrocatalytic effect of the quantum dots on the methylene blue electroreduction process. The genosensor could detect a synthetic single stranded DNA target in a range of 1.0×10^{-11} to 1.0×10^{-18} mol L^{-1} with a limit of detection of 2.0×10^{-19} mol L^{-1}, and genomic DNA in a range of 7.31×10^{-14} to 7.31×10^{-6} ng μL^{-1} with a limit of detection of 1.80×10^{-14} ng μL^{-1} with a high selectivity and sensitivity.

1. Introduction

Rapid, sensitive and simple determination methods of special gene sequence in very small sample volumes have attracted increasing attention in the diagnosis of genetic and infectious diseases, and detection of pathogenic microorganisms. Conventional DNA detection methods, i.e. PCR, gel electrophoresis and Southern blotting are labor intensive and time consuming [1–3]. Therefore, many techniques such as surface-enhanced Raman scattering [4], surface Plasmon resonance spectroscopy [5], fluorescence spectroscopy [6], UV–vis spectroscopy [7] and electrochemistry [8–11] have been developed for DNA detection. Among them, the electrochemical methods have recently received a good deal of attention due to the inherent specificity and sensitivity, their ease of use, low cost, fast response, and miniaturized and automated devices [8,9,12].

For many years, the appropriate modification of the conductive surfaces to immobilize biomacromolecules (such as DNA) has been one of the important approaches in biotechnology, bioanalysis, nanomedicine, and fabrication of biosensors [13–18]; it is also useful in understanding the action mechanism of drugs and origin of some diseases [19–21]. In a DNA biosensor, the immobilization of a DNA probe on the surface of an electrode is a key step and up to now, various kinds of nanostructured materials have been devised for the single-stranded DNA (ssDNA) immobilization [8,9,22,23]. This is due to their physicochemical properties to be highly tunable depending on their size and shape, unique surface chemistry, thermal stability, high surface area as well as good biocompatibility enabled by their nanometer sizes [12,24–28]. In this regard, magnetic nanostructures due to modulation in the chemical composition, size and magnetic susceptibility and good dispersibility are important for use in a variety of biosensing systems [29,30].

Cutaneous leishmaniasis is a group of protozoan infections caused by obligate intracellular protozoan parasites. These skin diseases occur in particular endemic regions in at least 88 countries of the world [31,32]. Annual incident of cutaneous leishmaniasis is estimated to be 1–1.5 million cases.

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Various laboratory methods are used to identify leishmaniasis, detect the parasite, and identify the *Leishmania* species [33–35]. Although epidemiological and clinical findings are necessary, they are not sufficient for identification of causative agents of cutaneous leishmaniasis. Diagnostic tests include light-microscopic examination of stained slides, in vitro culture from biopsy or lymph aspirate, Montenegro skin test, serological tests (including indirect fluorescent antibody test, enzyme-linked immunosorbent assay (ELISA) and isoenzyme analysis), and molecular tests (such as PCR). These methods are not sensitive enough or are indirect, require culture of the parasites or passage through an experimental animal which is time-consuming and sometimes unsuccessful. The antibody-based tests cannot detect early infections and distinguish between past and present infections, and cross-react with antibodies against other pathogens [36,37].

In the present study, quantum dots of magnetic cobalt-zinc ferrite ($Co_{0.5}Zn_{0.5}Fe_2O_4$) were synthesized and employed to modify a carbon paste electrode. A 24-mer specific sequence was immobilized to fabricate a label-free *Leishmania major* genosensor. The ability of the genosensor to detect *Leishmania major* in patient samples was investigated.

2. Experimental section

All chemicals were of analytical grade from Scharlau (Spain) or Sigma (USA) and were used without further purification. All solutions were prepared by redistilled water. A probe oligonucleotide (p-ssDNA) was designed based on the genomic sequence of *Leishmania major*. (p-ssDNA) was designed using NCBI BLAST nucleotide search tool and Primer3 based on a submitted sequence in GenBank (AB678349.1) of *Leishmania major* minicircle kDNA (Iranian Standard strain MCAN/IR/97/LON490, isolate: Iran JWmaj) [38]. The (p-ssDNA) sequence was evaluated for any possible homology and share sequences with any non-*Leishmania major* species using the BLAST of NCBI. In addition, the melting temperature of (p-ssDNA) was placed within a narrow range using Oligo analysis software. Finally, the (p-ssDNA) sequence was checked for the formation of potential self-dimer and secondary structures using mfold software (http://mfold. rna. albany. edu) [39] which may otherwise hinder the assay.

(p-ssDNA) was ordered from SinaClon BioScience Co. (Iran). A complementary-sequence oligonucleotide (target oligonucleotide, t-ssDNA) was purchased from SinaClon BioScience Co. (Iran). The oligonucleotide sequences were as follows:

(p-ssDNA) sequence: 5′-TGTTGGGTGACGCTTTAGTGGGTT-3′.

t-ssDNA sequence: 5′-AACCCACTAAAGCGTCACCCAACA-3′.

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

Quantum dots of magnetic cobalt-zinc ferrite ($Co_{0.5}Zn_{0.5}Fe_2O_4$) were synthesized by a precipitation route. Co(II), Zn(II) and Fe(III) chloride salts with a 1:1:4 stoichiometric ratios were dissolved in a 100 mmol L^{-1} hydrochloric acid solution and heated to 80 °C. Then, a solution of 4.0 mol L^{-1} of sodium hydroxide with a temperature of 80 °C was rapidly added to the salts solution and

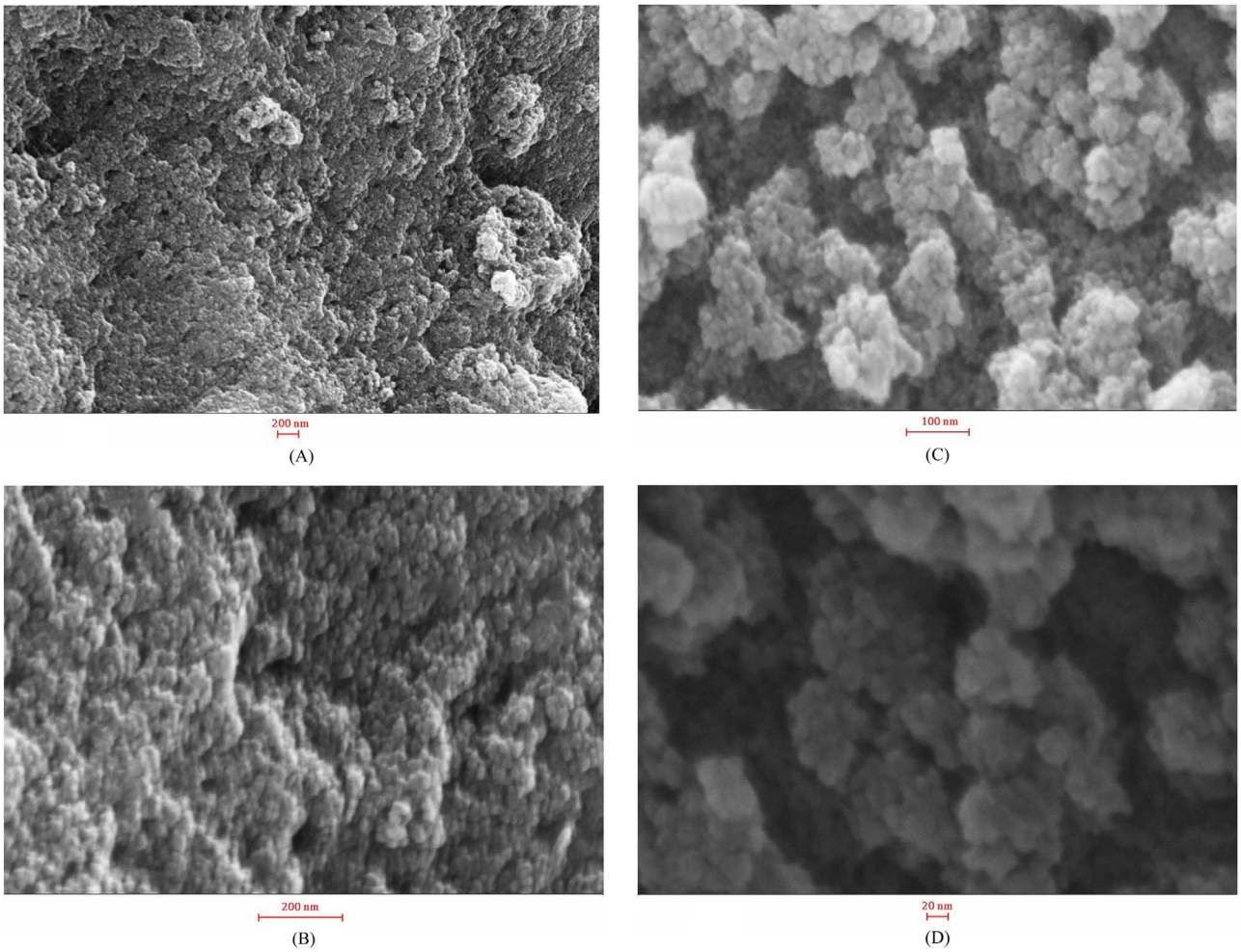

Fig. 1. FESEM images of magnetic cobalt-zinc ferrite quantum dots with different magnifications.