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An ultrasensitive electrochemical genosensor for *Brucella* based on palladium nanoparticles

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ABSTRACT

Palladium nanoparticles were potentiostatically electrodeposited on a gold surface at a highly negative potential. The nanostructure, as a transducer, was utilized to immobilize a *Brucella*-specific probe and the process of immobilization and hybridization was detected by electrochemical methods. The proposed method for detection of the complementary sequence and a non-complementary sequence was applied. The fabricated genosensor was evaluated for the assay of the bacteria in the cultured and human samples with and without PCR. The genosensor could detect the complementary sequence with a sensitivity of 0.02 μA dm^3 mol^{-1}, a linear concentration range of 1.0×10^{-12} to 1.0×10^{-19} mol dm^{-3}, and a detection limit of 2.7×10^{-20} mol dm^{-3}.

1. Introduction

DNA hybridization detection is a key parameter in the diagnosis of genetic diseases and pathogen agents. Various techniques such as immunoassays, surface plasmon resonance spectroscopy, quartz crystal microbalance, UV–vis spectroscopy [1–3] and electrochemical methods [4,5] have been developed based on the DNA hybridization detection. Electrochemical genosensors are one of the best choices for development of sensitive, specific, simple and inexpensive devices for disease diagnosis [6].

Brucellosis is an infectious disease caused by the gram-negative bacteria of the genus *Brucella* with an intracellular life cycle. Brucellosis infections are almost invariably transmitted to people by contact with animals or animal products that are contaminated with these bacteria [7]. *Brucella* organisms are also biological warfare agents [8]. The etiological agents of brucellosis are various *Brucella* species.

Bacterial culture is the important part in confirmation of the presence of this disease [9]. However, the growth rate of the bacterium is slow and fastidious leading to misleading results [10] with low sensitivity [11]; it is time consuming and poses a risk for laboratory personnel [12,13]. Other techniques have also been developed for brucellosis diagnosis such as complement fixation test (CFT), serum agglutination test (SAT), Rose Bengal plate test (RBT) [14,15], PCR, rtPCR and qPCR [16–18], up-converting phosphor technology-based lateral flow strip [19], monoclonal antibody-based assay [20], ELISA [21], real-time quantitative loop-mediated isothermal amplification assay (LAMP) [22], Raman spectroscopy [23], and serological tests [24]. Serological techniques suffer from serological cross-reactions [25,26], low sensitivity in the acute phase, low specificity and high prevalence of *Brucella*-specific antibodies in epidemic areas of brucellosis. PCR-based methods have been limitedly employed in human samples [27], need more expensive equipment, and suffer from difficulty in the direct detection of PCR products [19]. The LAMP method also needs six primers and gel electrophoresis step using intercalating dyes or fluorescent tags. Moreover, most of these methods have problems associated with them such as requiring sophisticated procedures, being time consuming and needing trained personnel. In addition, these methods are only adapted for the qualitative or semi-quantitative detection for *Brucella* assays, which do not meet the requirements for the rapid and accurate identification of brucellosis in a shorter time; it delays the introduction of efficient remedial measures. Some immunosensors for detection of *Brucella* have also

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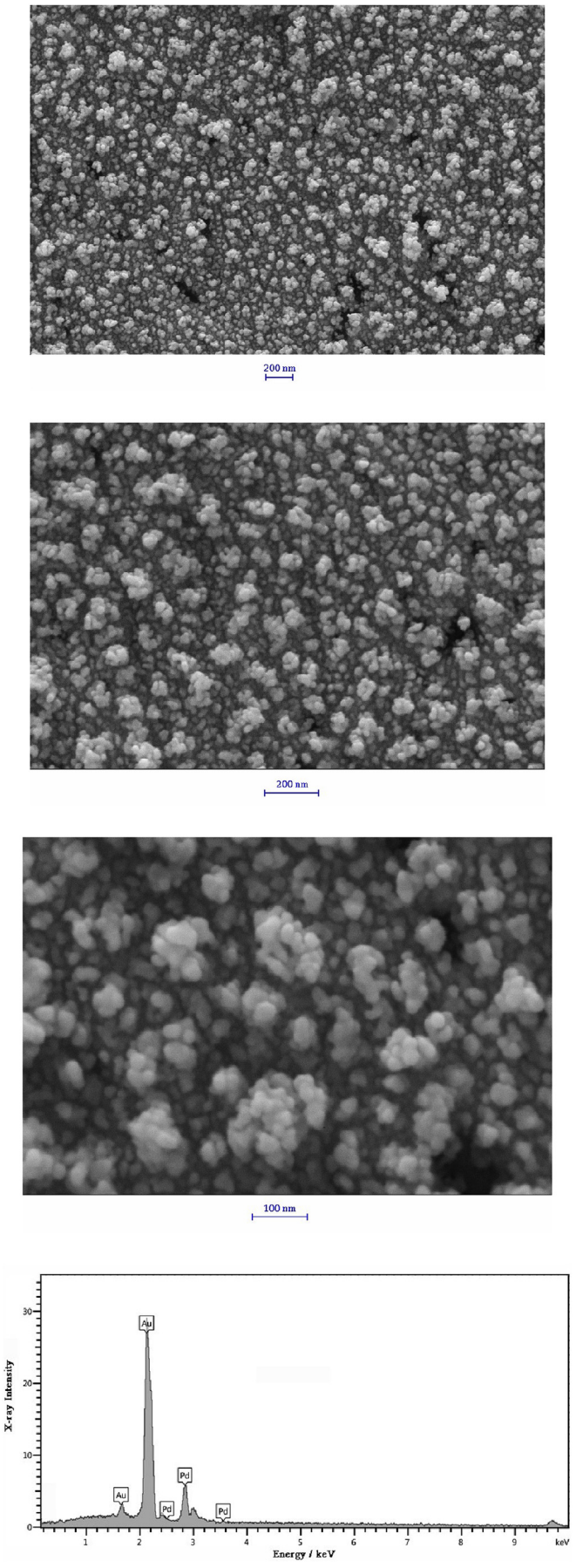

been reported [28,29]. However, most of these sensors are label-dependent and require labeling of biomolecules to convert the antibody/antigen interaction into detectable (optical or electrochemical) signals. Therefore, development of rapid, inexpensive, and easy-to-use methodologies for direct detection of *Brucella* directly in real samples has attracted considerable interest.

Applications of nanomaterials in biosensing provide the advantages of high surface area of the transducers, enhanced electronic properties and amplified readout systems, and electrocatalytic and biocompatible surfaces [30–34]. Palladium nanoparticles have thermal stability, chemical inertness and affinity for the thiol groups, and can be used as a sensing platform in genosensor fabrication.

In the present study, an electrochemical label-free genosensor was developed based on palladium nanoparticles as a transducer for a rapid, simple and quantitative detection of *Brucella* organism in clinical samples.

2. Materials and methods

2.1. Materials

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 thiolated oligonucleotide probe (probe oligonucleotide, p-ssDNA) was designed based on the common genomic sequence in all the *Brucella* species. The probe sequence was checked for any possible homology and share sequences with any non-*Brucella* species. Moreover, the melting temperature of the probe was ensured to be within a narrow range using Oligo software. Then, the probe sequence was checked for potential self-dimmer and formation of secondary structures using mfold software which may otherwise hinder the assay. p-ssDNA was ordered from Vivantis (Malaysia). A complementary-sequence oligonucleotide (target oligonucleotide, t-ssDNA) and non-complementary sequence oligonucleotide (nc-ssDNA) were purchased from SinaClon BioScience Co. (Iran). The oligonucleotide sequences are as follows:

p-ssDNA sequence: 5′ SH-$(CH_3)_6$ TGC CGA TCA CTT AAG GGC CTT CAT 3';
t-ssDNA sequence: 5′-ATG AAG GCC CTT AAG TGA TCG GCA-3′
nc-ssDNA sequence: 5′-AGA CCA AAA AGG CCA CCC CCG GGT-3′

The oligonucleotide stock solutions were prepared with 20 mmol dm^{-3} Tris–HCl buffer, pH 7.4 solution (Tris) and kept frozen.

2.2. Apparatus

Electrochemical measurements were carried out in a conventional three-electrode cell powered by a μ-Autolab potentiostat/galvanostat (the Netherlands). An Ag/AgCl, 3 mol dm^{-3} KCl, a platinum wire, and a palladium nanoparticles-modified gold disk (nPd) electrode (2 mm of diameter) were used as the reference, counter and working electrodes, respectively. The system was run on a PC by GPES 4.9 software.

DNA samples for PCR amplification were extracted from *Brucella* strains, *Staphylococcus aureus* and *Klebsiella*. The PCR reaction was performed on an Eppendorf Mastercycler Gradient PCR system

Fig. 1. FESEM images of the palladium nanoparticles with different magnifications and the corresponding EDS spectra.