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Development of sandwich-form biosensor to detect *Mycobacterium tuberculosis* complex in clinical sputum specimens

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ABSTRACT

Mycobacterium tuberculosis, the causing agent of tuberculosis, comes second only after HIV on the list of infectious agents slaughtering many worldwide. Due to the limitations behind the conventional detection methods, it is therefore critical to develop new sensitive sensing systems capable of quick detection of the infectious agent. In the present study, the surface modified cadmium-telluride quantum dots and gold nanoparticles conjunct with two specific oligonucleotides against early secretory antigenic target 6 were used to develop a sandwich-form fluorescence resonance energy transfer-based biosensor to detect *M. tuberculosis* complex and differentiate *M. tuberculosis* and *M. bovis* Bacille Calmette–Guerin simultaneously. The sensitivity and specificity of the newly developed biosensor were 94.2% and 86.6%, respectively, while the sensitivity and specificity of polymerase chain reaction and nested polymerase chain reaction were considerably lower, 74.2%, 73.3% and 82.8%, 80%, respectively. The detection limits of the sandwich-form fluorescence resonance energy transfer-based biosensor were far lower (10 fg) than those of the polymerase chain reaction and nested polymerase chain reaction (100 fg). Although the cost of the developed nanobiosensor was slightly higher than those of the polymerase chain reaction-based techniques, its unique advantages in terms of turnaround time, higher sensitivity and specificity, as well as a 10-fold lower detection limit would clearly recommend this test as a more appropriate and cost-effective tool for large scale operations.

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Introduction

Tuberculosis (TB) is one of the major chronic infectious diseases caused by *Mycobacterium tuberculosis* (MTB) and represents a global health concern.[1,2] It is posing a threat even in the developed countries, because it may emerge as an obstacle of human immune deficiency syndrome.[2] TB is spread in the air and can affect all parts of human body but mostly lungs.[3] MTB was reported to have caused 1.4 million deaths and 8.5 million incident cases across the world only in the year 2011.[3]

Beside MTB, the other members of what is commonly referred to as the *M. tuberculosis* complex including *M. africanum*, *M. microti*, and *M. bovis* may also cause TB infection. The only available vaccine against MTB is the attenuated *M. bovis* strains or Bacille Calmette–Guerin (BCG) which causes some cross-reactivity or false-positive results during the detection process.[1,4] Therefore, developing an accurate and reliable detection technique capable of differentiating infected samples from those vaccinated is also required.

On the other hand, early detection is so critical to avoid a TB epidemic. To achieve that, many techniques have been developed and widely applied to date such as bacteria's physical, physiological and biochemical characteristics as well as polymerase chain reaction (PCR)-based techniques.[2,5–11] More recently WHO recommended Xpert MTB/RIF detection technique as a primary sensitive diagnostic test.[12] However, all these techniques have a number of shortcomings and as a result, there is still a growing desire to accomplish a simple, rapid, sensitive and specific detection method to differentiate MTBC-infected samples from vaccinated samples with an affordable cost.[2,8,11,13,14]

The conserved genomic region of 6-kDa early secretory antigenic target 6 (ESAT-6) has been found of high homology among the different species of mycobacteria and has been used in the most studies to detect MTBC.[15,16] On the other hand, to differentiate BCG vaccinated samples from MTB-infected samples, the ESAT-6 genomic region which is eliminated in all available BCG strains but present in the MTB complex was utilized.[15,16] In addition, during the last decade, by applying nano-sized materials in biological detection and biological imaging aspects, the clinical diagnostics field has improved dramatically by developing rapid and sensitive methods at lower cost.[17,18] Semiconductor nanocrystals, also known as quantum dots (QDs), are one of the most attractive fluorescent nanoparticles which have been widely applied in bio-detection processes.[19] QDs are considered as alternative fluorescent probes for their unique optical properties such as high fluorescence yields, high photo-stability and narrow symmetric emission spectrum.[19] Moreover, the symmetric emission spectra of QDs have nominated them as an appropriate donor molecule for Förster resonance energy transfer (FRET)-based sensors,[18,20,21] in which the electronic excitation energy of a donor molecule is transferred to a nearby acceptor molecule via a dipole–dipole interaction between the donor/acceptor pair.[22] Case in point, the QDs broadly used as bio-sensors by immobilizing with particular probes to detect a specific target nucleotide.[23]

Gold nanoparticles (AuNPs) have also been utilized widely in the detection of specific RNA or DNA molecules due to their unique optical properties.[24,25] AuNPs have remarkably high extinction coefficients and a broad absorption spectrum, allowing higher sensitivity in optical detection techniques than traditional dyes and designating AuNPs as a suitable acceptor for FRET.[23,26] Due to the fact that, the emission and absorption spectrum of CdTe-QDs and AuNPs significantly overlap in 530 nm, the emission of the QDs is quenched when associated with oppositely charged AuNPs.[23,27]

In the present study, QDs were immobilized with a specific oligonucleotide (P1) and AuNPs was conjunct with another specific oligonucleotide (P2) against ESAT-6 region to develop a specific and sensitive sandwich-form FRET-based biosensor to detect and differentiate *M. tuberculosis* complex from BCG rapidly, accurately, and economically (Fig. 1). Moreover, the validity of sandwich-form FRET-based biosensor in comparison with culture, PCR and Nested PCR was evaluated.

Materials and methods

Samples

In the present study, 50 clinical samples (all subjects were HIV negative) were collected from sputum specimens of patients who were suspected to have tuberculosis in Tehran province hospitals, Iran (between December of 2005 and November 2008). The used procedures in the present study were in accordance with the ethical standards of the responsible committee on human experimentation from each participating hospital. The patients have given informed prior to participating in the research. The decontamination and cultivation of samples were carried out in the hospitals. Samples were decontaminated by using *N-acetylcysteine*–NaOH procedures. The mentioned samples were earlier analyzed and determined by using a cultivation technique as a gold-standard detection method by incubation 250 μL of *Mycobacterium* in the Lowenstein–Jensen media at 37 °C in humid atmosphere containing 5% CO_2.[28]

Primers and probes

In the PCR-based-detection phases, the primer sets were ordered based on an insertion sequence of the IS6110 gene region (Table 1).[29,30]

In the nano-based detection phase, two different oligonucleotide probes were used based on the conserved genomic regions of ESAT-6[16] to detect MTBC and differentiate between BCG and other *Mycobacterium* species simultaneously (Table 1). The 5′ end of the first oligonucleotide probe (P1) was linked to QDs by NH_2 and the 3′ end of the second oligonucleotide probe (P2) was linked to AuNPs by SH to facilitate the hybridization process. The probes were purchased from Invitrogen (Shanghai Invitrogen Biotechnique Co).

DNA detection by PCR and nested PCR assays

According to a DNA extraction technique described elsewhere,[31] the chromosomal DNA was extracted utilizing