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Pathogenic detection and phenotype using magnetic nanoparticle-urease nanosensor

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ABSTRACT

The diagnoses of bacterial infections remain a challenge in environment safety and human health area. Nanomaterial-based 'enzyme nose' sensor is a useful analytical technique for the detection of toxicologically important targets in biological samples. In this paper, we used three quaternized magnetic nanoparticles–urease sensors to detect bacteria with excellent sensitivity for several gram-positive or gram-negative bacteria. The response intensity of the nanoarray sensor is dependent on the level of displacement determined by three quaternized magnetic nanoparticles–urease binding strength and pathogenic cells–nanoparticles interaction. Based on the ability of urease to hydrolyze urea and increase the pH value of the system, the detection of pathogen is translated into a pH increase, which can be readily detected using a litmus dye. These characteristic responses of color show repeatable pathogenic cells and can be differentiated by principle component analysis (PCA). Our approach has been used to measure bacteria with an accuracy of 90.7% for 10^2 cfu mL^{-1} within 30 min. The measurement system has a potential for further applications and provides a facile and simple method for the rapid analysis of pathogens.

1. Introduction

The determination, identification, and quantification of pathogen are crucial for clinical diagnosis, environmental monitoring, and food safety control. It is important for analytical tools that the sensitive biological element, a biologically derived material or biomimetic component, interacts (binds or recognizes) with the analyte under investigation. Various sensitive, reliable, and rapid recognition elements, such as antibody, phage, lectin, Aptamers, bacterial imprint or cell receptor, have been reported for bacteria detection [1]. Antibodies, bacterial receptor and lectin are the most widely used biosensing elements to analyze pathogen due to their versatility of integration into biosensor [2,3]. Aptamers are single stranded nucleic acids that provide both strengths, such as low-cost and chemical stable, over antibody-based recognition element for bacterial detection [4,5]. However, several disadvantages presented by abovementioned bioelements, including variations of batch-to-batch, robustness in complicated substances, or relatively complex for preparation, have led to seek for alternative biosensing elements.

The 'chemical nose' approach is a newly developed technology for pathogen sensing, which employs multiple selective receptors that produce a response pattern for each target, allowing its classification. In this technique, a 'chemical nose' sensors are trained with qualified bacterial samples to set a reference database. Bacterial pathogen are then identified by comparing with the reference database. For example, Phillips et al. [6] developed a simple sensor based on hydrophobically functionalized gold nanoparticles which can quickly identify three different strains of *E. coli*. The negatively charged pathogenic cells competitively replaced the negatively charged conjugated polymers within the sensor system, which resulted differentially restoring the polymer fluorescence. Furthermore, the human's nose has been used to smell ultralow concentrations of volatile organic compounds present in complicated condition.

In another example, Duncan et al. [7]. took advantages of the selective recognition property of functionalized nanoparticles for bacterial sensing. The bacterial displace the lipase within the lipase-nanoparticle complex and reactivate the enzyme activity which amplify the signal response and allowed bacteria detection with short time (15 min) and high sensitivity (10^2 cfu mL^{-1}).

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The application of magnetic nanoparticles facilitate the separation, purification, and detection of pathogen from complicated matrixes. Magnetic nanoparticles are also ready to combine with various sensing platforms, such as fluorescent [8], electrochemical [9], Raman spectra [10], Mass Spectra [11], surface plasmon resonance [12], microcantilever [13], quartz crystal microbalance [14] and many other methods.

Previously, we reported an efficient magnetic nanoparticle based "chemical nose" for pathogenic detection. The bacterial membranes obstruct the q-MNP– fluorescent polymer, producing characteristics fluorescence response array. Through application of linear discriminant analysis, the response patterns were used to quantify different pathogens [15]. Additionally, based on amplification of response of functionalised magnetic nanoparticles, under an external magnetic field, we have fabricated a sensitive and reliable quartz crystal microbalance biosensor for detection of marine pathogen [16].

In this work, we designed three quaternized magnetic nanoparticles (qMNP)–urease nanocomposites for detection and identification of pathogen. The sensor was fabricated by non-covalent conjugation of qMNP with urease. The nanocomposites serve the dual functions of exhibiting differential affinities with bacterial cells and sensing the competitive binding to cell surfaces, resulting in rapid displacement of urease from the qMNP surface. The characteristic response pattern of nanoarray sensor can be readily detected using a litmus dye. To avoid the interference of qMNP–urease constructs in the measurement system, the nanocomplex can be separated quickly by a magnet.

2. Materials and methods

2.1. Instruments

Transmission Electron Microscopy (TEM) samples were prepared by depositing a diluted aqueous solution of *q*-MNP onto a carbon-coated copper grid. TEM images were observed on a JEOL JEM-2010 operated at 120 keV. ζ-Potential and dynamic light scattering (Malvern Zetasizer Nano ZS instrument) were used to characterize the charge and the hydrodynamic diameter of nanoparticles. The response values of nanoarray sensor were obtained from multimode microplate reader (Synergy NEO HTS, Bioteck).

2.2. Preparation of functionalized magnetic nanoparticles

In a typical synthesis of magnetic nanoparticles, a solution of 5 g of NaOH pellets in 250 ml of Millipore water was first purged with N_2 to remove oxygen and then heated to 40 °C. 100 ml of iron solution (6.48 g $FeCl_3{\cdot}9H_2O$ and 2.40 g $FeCl_2{\cdot}4H_2O$) was added dropwise using a syringe through a septum, and the mixture was stirred at 80 °C for 1 h. The black precipitate, magnetic nanoparticles (MNP), was washed four times by EtOH (100 ml) with sonication for 10 min.

MNP (1 g) was dispersed in EtOH (30 ml), with sonication for 20 min. To this reaction mixture, 2% (3-aminopropyl)-trimethoxysilane (10 ml) was added and continue sonication for 1 h. The product, amino-functionalized MNP (MNP-NH_2), was washed by dispersing in EtOH, followed by magnetic separation for three times.

MNP-NH_2 (1 g) was dispersed in EtOH (30 ml), with sonication for 20 min. To this reaction mixture, benzaldehyde or octanal (1 ml) was added and refluxed for 2 h. After this, sodium borohydride (100 mg) was added and further stirring of the mixture for 1 h. The product, N-derivated magnetic nanoparticles (N-MNP), was washed by dispersing in EtOH, followed by magnetic separation for three times.

N-MNP (1 g) in EtOH (30 ml) was added to NaOH solution (1.0 M, 10 ml). After 30 min of stirring at 50 °C, methylation was performed as follows: sodium iodide (2.2 g) and methyl iodide (22 g) were added to the N-MNP/NaOH mixture and then reacted for 24 h at 50 °C. The product, quaternized magnetic nanoparticles (q-MNP1 for methyl derivative, q-MNP2 for octanal derivative and q-MNP3 for benzaldehyde derivative), was washed by dispersing in EtOH, followed by magnetic separation for three times.

2.3. Inhibition of activity assay of urease with q-MNP

In the inhibition study, *q*-MNP and urease solutions were prepared in sodium phosphate buffer solution (pH 7.4). Then, urease (100 nM) was incubated with various concentrations of q-MNP 1, q-MNP 2 and q-MNP 3. Then the nanocomplex was removed using magnetic separation and the supernatant was added with 100 uL of a urea-containing solution (200 mM NaCl, 60 mM $MgCl_2$, 50 mM urea) and 10 uL of 0.04% phenol red. The activity of urease was followed by monitoring color change at 558 nm using a microplate reader (Bioteck, Synergy H1). The samples were measured in triplicate.

2.4. Bacterial cultivation

Bacteria were seeded and cultured in suspension using the following media: *S. aureus* (SA), *A. junii* (AJ), *V. harveyi* (VH), *M. luteus* (ML), *E. tarda* (ET), *V. Parahemolyticus* (VP) and *E. coli* (EC) in Luria–Bertani media. For this pathogen, a single colony was inoculated in bacterial medium and cultured at 37 °C for 12 h (150 rpm shaking). Before the assays, the bacterial were centrifuged at 8000 rpm for 5 min, washed with PBS, and diluted to desired concentration in PBS.

2.5. Detection of bacteria using urease/q-MNP nanocomposite

In the assay, urease (100 nM) was incubated with fixed concentrations of q-MNP1, q-MNP2 and q-MNP3 for 15 min. Then the nanocomplex was incubated with bacterial cells from 10^2 to 10^8 cfu mL^{-1} for 30 min and the supernatant was added with 100 uL of a urea-containing solution and 10 uL of 0.04% phenol red. The activity of urease was followed by monitoring color change for 15 min at 558 nm using a microplate reader. The samples were measured in triplicate. The raw response data matrix was processed by principle component analysis (PCA) using Data Processing System statistics v16.05.

3. Results and discussion

3.1. Fabrication of q-MNP/Urease nanosensor

To achieve rapid detection and phenotyping of bacteria, a MNP/Urease nanosensor array was designed for bacterial sensing. Initially, the synthesis of MNP was performed by co-precipitation method[17]. These small and superaramagnetic particles with high peak magnetization from this synthesis reaction can be functionalized directly with silanes in short time (1 h), and easily purified. The particles can further be functionalized. In this study, the amino-modified MNP was further derivated with methyl iodide, octanal, and benzaldehyde, and then quaternized. The q-MNP is associated with urease in aqueous solution to provide a pathogenic sensing system. Scheme 1 illustrates a representation of this bacterial detection method based on the q-MNP/Urease nanosensor. When pathogen is mixed with the MNP/Urease sensor system, the urease will be competitively replaced from the surface of the pathogen due to both electrostatic and hydrophobic interaction between nanoparticle with bacterial cells. In 2005, Berry et al. illustrated that