NANOPARTICLES-BASED ELECTROCHEMICAL BIOSENSOR FOR SINGLE BACTERIUM DETECTION BY REDOX SIGNAL AMPLIFICATION

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ABSTRACT

In this work, we report a portable microfluidic chip based on Si-Porous-Pt MS s(Mesoporous Silica nanoparticles) -enhanced electrochemical detection for single-bacteria diagnosis. The possibility of single-bacteria detection is realized here by using electroactive molecules modified on Si-Porous-Pt MS s(Mesoporous Silica nanoparticles) to detect and differentiate Staphylococcus aureus and Pseudomonas aeruginosa. The detection limit of these two electroactive molecules is near femtomolar (1 pM) when immobilized on Si-Porous-Pt MS s(Mesoporous Silica nanoparticles), and the minimum amounts of gold NPs required in the detection are around thousands (103 particle/μl). A concentration of 1000 cells/μl is detected by CV measurements. The linear detection limit concentration is form 1-1000 cells/μl. The results showed that the portable biochip system has great potential as a device for single-particle or possibly even single-organism detection.

KEYWORDS: Si-Porous-Pt MS s(Mesoporous Silica nanoparticles); single bacteria detection; redox signal amplification

INTRODUCTION

Conventionally, bacteremia diagnosis relies on bacteria culture and its subsequent biochemical characterization that may take several days to complete. Therefore, rapid, sensitive, simple-to-operate detection methods for bacterial pathogen detection in whole blood are in urgent demand. However, direct detection of a few live bacteria in many industrial or clinical samples is still very challenging. One common method to enable the few bacteria detection is by using Si-Porous-Pt MS s(Mesoporous Silica nanoparticles) to enhance the electrochemical characteristics of captured bacteria [1-2]. In electrochemical detection, the challenge is how to have enough signals from electrochemical reactions over background noise from medium for detection [3-4].

Bacterial infections remain a major threat to human health despite the recent advancement in medical diagnostic and therapeutic technologies. Bacteria invasion into human circulation, also called bacteremia, commonly results in serious outcomes, including death, in the affected individual. Currently, bacteremia diagnosis relies on bacteria culture and its subsequent biochemical characterization that may take several days to complete. Therefore, rapid, sensitive, simple-to-operate detection methods for bacterial pathogen detection in whole blood are in urgent demand. This proposal plans to integrate advanced nanomaterial-based diagnostic reagents with state-of-the-art microfluidic device to generate a novel whole-blood pathogenic bacteria diagnostic system, targeting to detection and differentiation of three major nosocomial bacterial pathogens: Staphylococcus aureus, Pseudomonas aeruginosa, and Klebsiella pneumoniae. The purified bacteria will then be mixed with specific antibodies conjugated with Si-Porous-Pt MS s(Mesoporous Silica nanoparticles) previously functionalized with electrochemically active molecules. Three different electrochemical active molecules will be used for differentiation of different bacterial species. The bacteria bound with the Si-Porous-Pt MS s(Mesoporous Silica nanoparticles) will be further separated from free Si-Porous-Pt MS s(Mesoporous Silica nanoparticles) using a valve made of carbon nanotubes and enter the detection chamber. The detection chamber is designed to equip five independent antibody-based, electrochemical detection areas, each for a bacterial pathogen. Overall, the chip will have many properties suitable for point-of-care uses, including rapid, sensitive, simple to operate, and a small portable size.

To achieve single bacterium detection, the key is to enlarge and fully collect reaction signals from the reactions. As a result, in this study, Si-Porous-Pt MS s(Mesoporous Silica nanoparticles) labeled with antibody and electroactive molecules were used as transducers to enhance signal. On the other hand, a C Ts filter valve for molecule concentration and separation is adopted here for bacteria concentration [5]. By combination of these two techniques, few to single molecules can be detected without amplification.

EXPERIMENTAL

The operation procedure and detection principle of this device is shown in fig. 1. Purified bacteria were mixed with specific antibodies conjugated Si-Porous-Pt MS s(Mesoporous Silica nanoparticles) previously functionalized with electrochemically active molecules (fig. 1a). First, we configure the different sizes of nano-level batch, additional concentration, the electrochemical signals of different types of molecules and the autonomy of monolayer. Then, in our laboratory, electrochemical analyzer to measure the electrochemical signal to get a ball on the electrochemical signal elements can be connected and the autonomy of the best ratio of single-molecule film. The ball will be dropping in whole blood, by the antigen on the ball to catch bacteria to bacteria signal amplification effect. Suppose a bacterium can be connected on the ball 104, while a ball and is...
connected on the electrochemical activity of 102 molecules, then we can expect a bacterial signal to the original can be enlarged to 106 times, so even if by signal amplification a small number of bacteria can be detected. We designed the experimental device characteristics are:

1. o electrochemical transfer media.
2. The use of ball can effectively enhance the signal. 3. This is a new type of bacterial detection method

We use external signals to the bacteria, the bacterial signal different from the traditional way of direct measurement. Present experiments the optimal conditions obtained: 30 nm ball the size of the solution for the ball with the electrochemical activity of 1μM and 1mM autonomous molecular monolayer (SAM), this condition can be the strongest signal measured electrochemical signal.

![Figure 1](image1)

**Figure 1**: The chip design and biosensing mechanism. (a) Surface modification process of Si-Porous-Pt MSNs(Mesoporous Silica Nanoparticles), (b) Incubation and filtration processes of gold NPs when binding on bacteria, (c) Principle of electrochemical detection and bacteria capture

**Figure 2**: The microfabrication processes of electrochemical immunosensor microfluidic chip.

![Figure 2](image2)

**Figure 3**: Silicon nanospheres electrochemical bonding molecules can detect the saturation concentration

**Figure 4**: Electrochemical bonded silicon nanospheres molecule Linearly concentration

![Figure 4](image3)

**Figure 5**: Calculating the minimum required single bacterial Mesoporous Silica Nanoparticles concentration

![Figure 5](image4)

![Figure 6](image5)

![Figure 6](image6)
RESULTS AND DISCUSSION

The bacteria bound Si-Porous-Pt MSs (Mesoporous Silica nanoparticles) will be further separated from free Si-Porous-Pt MSs (Mesoporous Silica nanoparticles) (figure 1b) using an array of carbon nanotubes filter (figure 2a) and enter the detection array electrodes (figure 1c, 2b). The electrodes are designed to equip two to three independent antibody-based, electrochemical detection areas, each for a bacterial pathogen which was captured by specific antibody modified on electrode surface (figure 2b). Two to three different electrochemical active molecules will be used for differentiating different bacterial species. The microfabrication processes of electrochemical immunosensor microfluidic chip was shown in figure 3 (a)-(l). In figure 4, during the incubation and separation, the target bacteria which were conjugated with modified Si-Porous-Pt MSs (Mesoporous Silica nanoparticles) are confined at the edge of chamber while the non-conjugated nanoparticles passing through the pillar structured CNTs valve (figure 2d). There are two fluor-dye labeled anti-antibodies used to distinguish Staphylococcus aureus and Pseudomonas Aeruginosa before and after incubation (figure 4a-4d). Figure 5 shows the oxidation and reduction signals of (A), 3-Amino-1,2,4 thriazole-5-thiol, (B), 5-Amino-2-mercaptobenzimidazole in illustration. At the condition of concentration limits of electroactive molecules (1 pM, 105 mole/μl, gray line), thousands (∼1500 particle/μl) of electroactive molecules labeling Si-Porous-Pt MSs (Mesoporous Silica nanoparticles) are needed for gated the equal sensing signal, and each bacterium may contains 75-120 Si-Porous-Pt MSs (Mesoporous Silica nanoparticles) by TEM estimation. As a result, the detection of about 10-20 bacteria in the current device has been realized, revealing a potential of this device for few to single bacteria detection. The Figure 6. shows the linear concentration detection limit of three different electrochemical active molecules (A: 4-pentyl-thiazole-2-thiol, B: 3-Amino-1,2,4 thriazole-5-thiol, C:5-Amino-2-mercaptobenzimidazole) for K. pneumoniae, S. aureus and P. Aeruginosa sensing. The Fixed concentration of bacterial samples were diluted configured 1,10,100,1000 (cells / μl) concentrations, measuring the concentration of the electrochemical signal over time, while taking advantage of the ball on the TEM number of bacteria taken, using the formula ($S = 6000 / \rho d$) calculated that single cells can be obtained about 100 ball, as shown in Figure 2e. These results showed that this portable biochip system has great potential as a device for single-particle or possibly even single-bacteria detection.

REFERENCES


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