Preparation of Functional Magnetic Nanoparticles Mediated with PEG 4000 and Application in *Pseudomonas Aeruginosa* Rapid Detection

Yongjun Tang¹,²,†, Zhiyang Li³,⁴,†, Nongyue He¹,⁴,∗, Liming Zhang¹,⁴, Chao Ma¹, Xiaolong Li¹,⁴, Chuanyan Li¹, Zhifei Wang¹, Yan Deng⁴, and Lei He³

¹State Key Laboratory of Bioelectronics, Southeast University, Nanjing 210096, China
²School of Chemistry and Chemical Engineering, Hunan Institute of Engineering, Xiangtan 411104, China
³Nanjing Longliang Biological Science Technology Co. Ltd., Nanjing 210000, China
⁴Hunan Key Laboratory of Green Packaging and Application of Biological Nanotechnology, Hunan University of Technology, Zhuzhou 412007, China
⁵College of Life Science, Yangtze University, Jingzhou 434025, China

A rapid detection method of *Pseudomonas aeruginosa* based on magnetic separation and chemiluminescence was developed in this paper. Magnetic nanoparticles (MNPs) were prepared by solvothermal method with PEG-4000 as a surfactant, and then were modified. The prepared MNPs present a uniform morphology and good dispersion. The sizes of MNPs can be controlled by adjusting the dosage of FeCl₃·6H₂O. The obtained particles were characterized with Scanning electron microscope (SEM), Transmission electronic microscopy (TEM) and Fourier transform infrared (FTIR). The biotin-dUTP-labeled DNA fragments of gyrB gene were amplified by polymerase chain reaction (PCR), and *Pseudomonas aeruginosa* was successfully detected with detection limit as low as 7.5 fM of gyrB fragments.

**Keywords:** Magnetic Nanoparticles, *Pseudomonas Aeruginosa*, Surface Modification, gyrB, Chemiluminescence.

Nanotechnology is one of the most important technologies in the past decades. The magnetic nanoparticles have been widely applied in the detection of various biological signals because of that they can be easily collected under the external magnetic field and, furthermore, they have the characteristics of high surface area.¹⁻⁶ Since the applications of magnetic nanoparticles (MNPs) in biotechnology and biomedical areas, much attention has been paid to the synthesis of different kinds of MNPs.⁷⁻⁹

*Pseudomonas aeruginosa* is a common pathogen to people and animals which can cause wounds infected and festering. Although *Pseudomonas aeruginosa* is not a kind of strong-pathogenic bacteria, but it is an opportunistic pathogen and is easy to get resistance from the outside world, so *Pseudomonas aeruginosa* have tenacious vitality.¹⁰⁻¹² The infection of *Pseudomonas aeruginosa* is one of the main reason why the patients get septicemia after they receiving organ transplants. According to the statistics of Khan et al. in 1994, about 28% of sepsis were caused by *Pseudomonas aeruginosa*.¹³,¹⁴ *Pseudomonas aeruginosa* also is a major cause of morbidity and mortality in patients with cystic fibrosis (CF). Research shows that, in order to prevent and delay the occurrence of CF, early effective antibiotic treatment is very important. Therefore, building rapid and sensitive early detection technology of *Pseudomonas aeruginosa* is very important and necessary.¹⁵⁻²⁰

In this paper, Fe₃O₄ nanoparticles were prepared by solvothermal method with surfactant PEG-4000 which was first used in the preparation of MNPs. The MNPs were then modified for application in the detection of pathogen. The carboxyl-modified MNPs were conjugated with amino-modified probe by incubation, and then the probe-modified MNPs were used in detection of specific gyrB gene of *Pseudomonas aeruginosa* which was amplified with biotin-labeled-dUTP. Finally, we developed a rapid detection method of *Pseudomonas aeruginosa* based on magnetic separation and chemiluminescence.²¹⁻²³

*Pseudomonas aeruginosan* strain (ATCC27853) was purchased from Guangdong Huankai Microbial Sci. and
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The amino-modified gyrB probe (5′-CCGTGGTGAGACCTGTTCCACACC-(15T)-NH2-3′) and primer pair (5′CCTGACCATCCGTCGCCAACAC3′ and 5′CCTGACCATCCGTCGCCACAC3′) were synthesized and HPLC purified by the Sangon Company (China). 3-(2′-spiroadamantane)-4-methoxy-4-(3′-phosphoryloxy) phenyl-1,2-dioxetane (AMPPD) was purchased from the Biochem-ZX (China). Streptavidin-modified alkaline phosphatase (SA-AP) and PEG-4000 was purchased from the Sangon Company (China). The biotin-11-dUTP was purchased from the Fermentas (USA). The particle size and morphology of the nanoparticle samples were determined by Scanning electron microscope (SEM) with Zeiss Ultra Plus, Transmission electronic microscopy (TEM) with JEM-200CX, and Fourier transform infrared (FTIR) with Nicolet 5700.

Fe3O4 nanoparticles were prepared by the solvothermal method. 2.03 g of FeCl3·6H2O, 2 g of PEG-4000 and 4 g of NaAc were dissolved in glycol. The solution was placed in a reaction kettle and allowed to react for 12 h in 180 °C. The obtained precipitate was washed by deionized (DI) water for 3 times. Fe3O4@SiO2 nanoparticles were prepared according to the method as follows. Firstly, the obtained nanoparticles were dispersed in 150 mL solution of ethanol/water (4:1) with the help of the ultrasonic. 3 mL of tetraethoxysilane (TEOS) and 4 mL of ammonia were added into the mixture respectively, and then the obtained mixture was stirred for 3 hours. Finally, the product was separated from the reactant mixture by the external magnetic field, and washed by ethanol for 3 times. The amino-modification of Fe3O4@SiO2 was performed according to the method by Li et al. 6.7 mL of Fe3O4@SiO2 MNPs were separated by the external magnetic field and were then dispersed in 30 mL of ethanol/water (199:1) solution by ultrasonic, and then 60 μL of 3-aminopropyl triethoxysilane (APTES) was added into this mixture. The solution was stirred for 7 h at room temperature. The product was separated from the reactant mixture by the external magnetic field, and washed with ethanol for 5 times and N,N-dimethylformamide (DMF) for 5 times, respectively. The amino-modified MNPs were dispersed in DMF to a concentration of 50 mg/mL. For carboxyl-modification, 600 μL of amino-modified MNPs solution prepared as above were dropped into equal volume of succinic anhydride solution, and the mixture was stirred at 20 °C for 24 h. The carboxyl-modified MNPs were then acquired by magnetic separation after washed with DI water for 3 times. The probe was modified on the surface of magnetic nanoparticles according to the previously reported approach. The probes were...
designed to capture the biotin-dUTP-labeled DNA fragments which were obtained by PCR. Then these complexes were bonded with SA-AP. Finally the chemiluminescent signals were detected by adding AMPPD which was the substrate reagent of AP. The data were processed using SPSS 13.0 software.

The surface morphology and particle size of the obtained MNPs are shown in Figure 1. The particles (A) possessing a size in the range of 250–400 nm were prepared with 2.03 g of FeCl$_3$·6H$_2$O, while the particles (B) prepared with 0.68 g of FeCl$_3$·6H$_2$O present a smaller size in the range of 180–300 nm. Therefore, the size of particles can be controlled in this method by adjusting the dosage of FeCl$_3$·6H$_2$O. It can be found that the two kinds of MNPs are spherical with uniform shape.

For the prepared Fe$_3$O$_4$@SiO$_2$ core–shell particles, the SEM and TEM images are shown in Figures 2(A) and 3. Compared with the naked Fe$_3$O$_4$ particles shown in Figure 1, the surface of Fe$_3$O$_4$@SiO$_2$ core–shell nanoparticles became much smoother owing to the coating by silica. The TEM images showed a very clear core–shell structure, and the shell of silicon is very thin which offers good

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**Fig. 2.** SEM images of the surface modified magnetic nanoparticles. (A) The Fe$_3$O$_4$@SiO$_2$ nanoparticles. (B) The carboxyl-modified Fe$_3$O$_4$@SiO$_2$ nanoparticles.

**Fig. 3.** TEM images of the Fe$_3$O$_4$@SiO$_2$ nanoparticles.
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These nanoparticles have uniform shape and good dispersion property, and each nanoparticle has one single core. From Figure 2(B), it can be observed that the carboxyl-modified MNPs also have uniform shape and good dispersion. The FTIR spectra for the Fe$_3$O$_4$ nanoparticles and Fe$_3$O$_4$@SiO$_2$ nanoparticles were showed in Figure 4. The Fe$_3$O$_4$@SiO$_2$ nanoparticles have a strong absorption band at about 1100 cm$^{-1}$, and two weak absorption bands at about 900 cm$^{-1}$ and 800 cm$^{-1}$. These bands can be ascribed to the symmetric and asymmetric stretching vibration of framework and terminal Si–O–Si groups, and the bands at about 3400 cm$^{-1}$ are absorption of O–H.$^{34,35}$ The spectra could prove that the Fe$_3$O$_4$ nanoparticles have been coated by silica.

Finally, the carboxyl-modified Fe$_3$O$_4$@SiO$_2$ nanoparticles were applied in the detection of Pseudomonas aeruginosa after they were conjugated with amino-modified gyrB probe. The biotin-dUTP-labeled DNA fragments of gyrB gene were amplified by PCR. As shown in Figure 5, the strip of lane (A) is the gyrB gene fragments (222 bp) and the strip of lane (B) is an universal gene fragments (206 bp) as control. After the MNPs-probes were hybridized with gyrB gene fragments, SA-AP was added into the products. The chemiluminescent intensity was detected by adding AMPPD as substrate. To test the sensitivity of the system for detecting gyrB of Pseudomonas aeruginosa, the gyrB fragments of different content were detected, and the result was presented in Figure 6. It was showed that the chemiluminescent intensity of sample increased by 39.8% ($P < 0.01$ vs. control) when the content of gyrB fragments is 7.5 fM in sample. However, the difference shows no statistical significance when the concentration of gyrB fragments is lower than 3.75 fM.

In conclusion, the MNPs were prepared using PEG-4000 as surfactant which was first applied in the preparation of MNPs. It was proved that the as-prepared MNPs have uniform shape and good dispersion, and they were also successfully modified with –NH$_2$ and carboxyl, while still remaining uniform shape and good dispersion. Furthermore, a rapid detection method for Pseudomonas aeruginosa was successfully established. The results showed that the chemiluminescent intensity of sample increased by 39.8% ($P < 0.01$ vs. control) when the content of gyrB fragments is 7.5 fM in sample. However, the difference shows no statistical significance when the concentration of gyrB fragments is lower than 3.75 fM.

Fig. 4. The FTIR spectrum of the modified magnetic nanoparticles. (A) The Fe$_3$O$_4$ nanoparticles. (B) The Fe$_3$O$_4$@SiO$_2$ nanoparticles.

Fig. 5. The agarose gel electrophoresis of the gyrB gene fragments (A) containing biotin-11-U and the universal gene fragments (B) containing biotin-11-U.

Fig. 6. The sensitivity analyse of Pseudomonas aeruginosa detection based on the as-modified MNPs and chemiluminescence. (A) PCR products were tested after the gyrB fragments were diluted to 30 pM, 3 pM, 0.3 pM, 0.03 pM. (B) PCR products were tested after the gyrB fragments were diluted to 30 fM, 22.5 fM, 15 fM, 7.5 fM, 3.75 fM. $^*$ $P < 0.05$ versus control; $^{**}$ $P < 0.01$ versus control.

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It seems that the results from this study will bring some new ideas to related societies.

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References and Notes

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