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Short communication

Rapid pathogen detection with bacterial-assembled magnetic mesoporous silica

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ABSTRACT

We report rapid and accurate pathogen detection by coupling with high efficiency magnetic separation of pathogen by Ni^{2+}-heterogeneous magnetic mesoporous silica (Ni-HMMS) and real time-polymerase chain reaction (RT-PCR) technique. Ni-HMMS was developed with a significant incorporation of Fe particles within the silica mesopores by programed thermal hydrogen reaction and functionalized with Ni^{2+} ion on the surface by the wet impregnation process. High abundant Ni^{2+} ions on the Ni-HMMS surface were able to assemble with cell wall component protein NikA (nickel-binding membrane protein), which contains several pathogenic bacteria including *Escherichia coli* O157:H7. NikA protein expression experiment showed the outstanding separation rate of the *nikA* gene-overexpressed *E. coli* (pSY-Nik) when comparing with wild-type *E. coli* ($44.5 \pm 13\%$) or not over-expressed *E. coli* (pSY-Nik) ($53.2 \pm 2.7\%$). Moreover, Ni-HMMS showed lower obstacle effect by large reaction volume (10 mL) than spherical core/shell-type silica magnetic nanoparticles functionalized with Ni^{2+} (ca. 40 nm-diameters). Finally, the Ni-HMMS was successfully assessed to separate pathogenic *E. coli* O157:H7 and applied to direct and rapid RT-PCR to quantitative detection at ultralow concentration (1 Log_{10} cfu mL^{-1}) in the real samples (milk and *Staphylococcus aureus* culture broth) without bacterial amplification and DNA extraction step.

1. Introduction

Nucleic acid analysis is a preferable approach to detect pathogens because it provides accurate detection of things such as pathogen's virulence, antibiotic resistance, and epidemiology (Belgrader et al., 1999; Stöhr et al., 2005; Zelada-Guillén et al., 2009). Real time-polymerase chain reactions (RT-PCR) are the most reliable and accurate detection methods since detailed genetic information about particular pathogen can be obtained at the nucleic acid level. On the other hand, a potential issue is the collection of pathogens and entire genome extraction from uncultured samples in order to enhance PCR sensitivity (Labrenz et al., 2004; Justé et al., 2008). In terms of advancing detection sensitivity, high efficiency separation of pathogens at ultralow concentrations is an important step in pre-treatment of RT-PCR analysis.

Recently, magnetic separation has enabled the development of new diagnostic platforms allowing greater sensitivity and swifter detection (Lee et al., 2009; Kang et al., 2009). Current research has focused on mesoporous silica (MS) structures due to their excellent physical properties of large specific surface area, fine pores, and high stability, which are beneficial for use as catalysts, separators, and adsorbents (Yamauchi et al., 2009; Sun and Bao, 2008; Qiao et al., 2012). In order to magnetize these mesoporous silica, an early study on magnetic mesoporous silica (MMS) preparation involves incorporation of magnetic Fe_xO_y nanoparticles (MNPs) within the mesopores (backfilling) and embedding of MNPs in the mesoporous walls (Shylesh et al., 2010; He and Antonelli, 2002). However, these approaches are limited posing a serious problem for high magnetization; for instance, clogging of the matrix pores decreases the surface reactivity and magnetic susceptibility (Garcia et al., 2003). Thus, mesoporous silica with high Fe concentrations should be considered a new class of mesoporous silica with high-performance magnetization.

By employing elemental iron (Fe^{2+}) as a Fe precursor, we developed a straightforward temperature-programmed reduction route towards a heterogeneous structure of high Fe content MMS (HMMS) (Kang et al., 2010; Lee et al., 2013). Subsequently, the synthesized HMMS were functionalized with nickel ion (Ni^{2+}) in order to provide stronger hydrogen bonding with pathogen cell wall components (Fig. 1A) Ni^{2+} is known to be an essential cofactor in several bacterial enzymes (Cavazza et al., 2011). The virulence of several pathogens, including *Helicobacter pylori* and *Vibrio parahaemolyticus*, are dependent on the nickel-binding membrane protein named NikA (Park et al., 2000). The biochemical function of NikA, a transmembrane protein, has been identified in *Escherichia coli*

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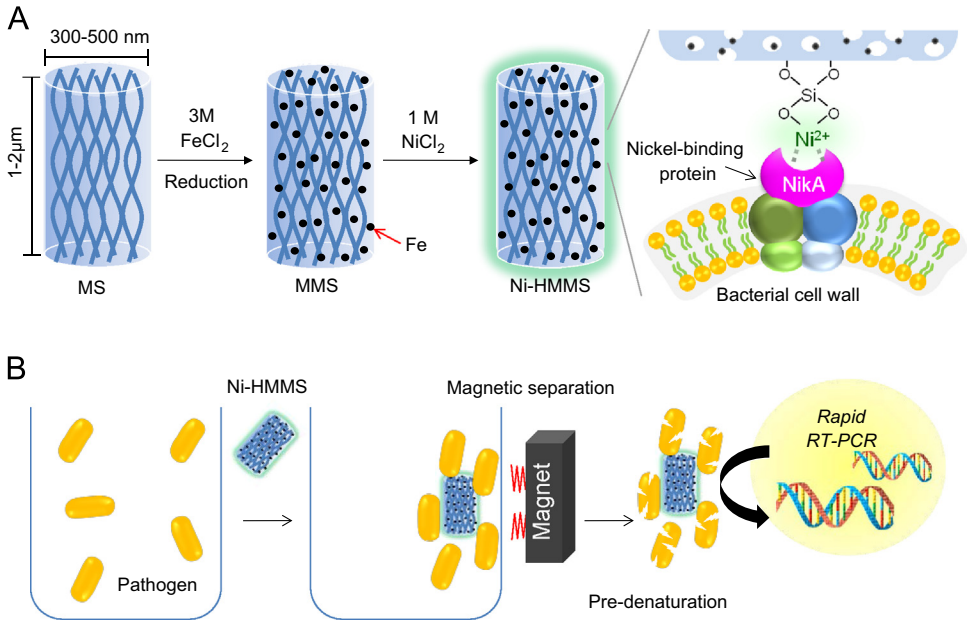

Fig. 1. (A) Schematic representation of the Ni-HMMS preparation and possible conformational interactions between the Ni-HMMS and bacterial cell wall and (B) detection process of pathogens by Ni-HMMS and rapid RT-PCR.

among a number of accessory proteins in bacterial cell wall nickel ion import systems (Heddle et al., 2003; Hiron et al., 2010). Therefore, the NikA of a pathogen and Ni^{2+} of a HMMS (Ni-HMMS) would bind and allowing the pathogen to be magnetically separated from water.

Herein, we report a new approach for rapid and accurate pathogen detection through connecting high efficiency magnetic separation of bacteria with molecular genetic method. For the purpose, we developed a HMMS structures with a significant incorporation of Fe particles within the silica mesopores by programmed thermal hydrogen reduction. Furthermore, Ni^{2+}-participation on the HMMS surface enables efficient and facile separation of *E. coli* O157:H7 as a model pathogen at ultralow concentration from a water sample and helps to amplify subsequent detection processes by direct RT-PCR analysis (Fig. 1B).

2. Experimental section

2.1. Materials

Pluronic P123 ($PEO_{20}PPO_{70}PEO_{20}$) was purchased from BASF (Korea Branch). Tetraethyl orthosilicate (TEOS, 99.9%), iron (II) chloride ($FeCl_2 \cdot 6H_2O$) and nickel (II) chloride hexahydrate ($NiCl_2 \cdot 6H_2O$) were purchased from Aldrich. All other chemicals were of extra pure analytical grad and were used without further purification. All aqueous solutions were prepared with double-distilled water, obtained from a Milli-Q water purifying system (18.3 MΩ cm).

2.2. Preparation of the Ni^{2+}-functionalized HMMS

The mesoporous silica (MS) with a 2D-hexagonal structure was prepared using the triblock co-polymer Pluronic P123 ($PEO_{20}PPO_{70}PEO_{20}$) as a surfactant template. MS structure with ordered shape was prepared with 4.0 g of Pluronic P123 dissolved in 30 g of water and 120 g of 2 M HCl and stirred at 40 °C for 8 h. The 8.5 g of tetraethyl orthosilicate (TEOS) was added into the clear solution, and stirred at 40 °C for 8 h. The reaction mixture was transferred to a Teflon-stainless steel bomb for hydrothermal treatment at 120 °C for overnight. The resulting white solid was filtered and washed with H_2O and air-dried. The surfactant template was removed by the calcination in air at 550 °C for 8 h. Iron (II) chloride ($FeCl_2 \cdot 6H_2O$) was used as the iron precursor. 5 g of MS was suspended in 3 M $FeCl_2 \cdot 6H_2O$. To synthesis HMMS, the mixture was stirred for 2 h and dried under vacuum for 12 h, and then reduced in H_2 flow (3 mL/min) at 400–600 °C for 2 h. 1 M nickel (II) chloride hexahydrate ($NiCl_2 \cdot 6H_2O$) was introduced into pore and surface of HMMS by wet impregnation process to prepare Ni-HMMS.

2.3. Bacterial strains and growth conditions

Bacterial strains and plasmids used in this study are all listed as follow; *E. coli* O157:H7 (ATCC 43888; American Type Culture Collection), *Staphylococcus aureus* (ATCC 33592), and *E. coli* BL21 (DE3) (Novagen Chemicals, Inc., Germany) cells were grown at 37 °C in Luria-Bertani medium. Selection for the presence of plasmids was carried out by using ampicillin (50 μg mL^{-1} for *E. coli* strain). Shake flask culture growth was monitored by measuring the optical density at 600 nm (OD_{600}) using a UV/vis spectrophotometer (Jasco Co., Japan).

2.4. Construction of recombinant plasmid for NikA overexpression in E. coli

The *nikA* coding region was amplified from the chromosomal DNA of *E. coli* BL21 (DE3) by polymerase chain reaction (PCR) using the primer pair nikA-F (5′-ATT GGA TCC ATG CTC TCC ACA CTC CGC-3′, *BamH*I) and nikA-R (5′–GTG AAG CTT TTA AGG TTT CAC CGG TTT-3′, *Hind*III). The PCR product was digested with *BamH*I/*Hind*III and ligated into the *BamH*I/*Hind*III-restricted plasmid, pET-21a. The resulting plasmid pSY-Nik was transferred to *E. coli* BL21 (DE3) by $CaCl_2$ transformation. The NikA protein was induced in recombinant *E. coli* BL21 carrying pSY-Nik by the adding of 0.5 mM IPTG after the culture had reached an optical density at 600 nm of 0.6, and then the cells were grown for 3 h. Overexpressed proteins were analyzed by SDS-PAGE and their concentrations were estimated by standard bicinchoninic acid protein assay using Pierce BCA Protein Assay kit (Thermo Scientific Inc., USA).