Silica coated Magnetic Nanoparticles (SMNPs): Capture and Identification of *Escherichia coli* Cells using Surface Enhanced Raman Spectroscopy

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**ABSTRACT**

Silica coated Magnetic nanoparticles (SMNPs; SiO$_2$/Fe$_3$O$_4$ + Fe$_2$O$_3$; 75±10 nm in diameter) were prepared by encapsulation of iron oxide NPs with silica dioxide using the sol-gel method and characterized through transmission and scanning electron microscopic imaging, magnetic hysteresis and Fourier transform infrared spectroscopy. The hydroxyl group of SMNPs surface were chemically activated with cyanogens bromide and then functionalized with specific polyclonal antibodies. The functionalized NPs were used to capture and concentrate the environmental *Escherichia coli* (*E. coli*) from several local environmentally impacted water streams. The capture of bacteria was confirmed by plating on nutrient agar. Raman spectroscopy was used for detection and monitoring of bacterial disintegration in presence of silver nanoparticles (6±4 nm). Raman spectra of bacterial complex shows the various characteristics biomolecules, this confirms the detection of *E. coli* captured by SMNPs. This method may ultimately be use as a rapid monitoring procedure for water quality.

**Keywords:** magnetic silica nanoparticles, *Escherichia Coli*, TEM & SEM, SERS

1 INTRODUCTION

The whole cell identification of microorganisms is critical for the food – safety and environmental protection. There are number of conventional method are available for microbial identification [1]. However, most of them are laborious and expensive. The spectroscopic methods like fluorescence [2], mass (MALDI) [3], IR [4] and surface-enhanced Raman spectroscopy (SERS) [5] are currently used to save time. These techniques are capable to identifying a whole microorganism from a limited number of microbial cells in a non-destructive manner.

Among, the SERS has drawn attention because it is compatible with biological samples, requires lesser sample preparation and provide signals with detailed information concerning microorganism [6]. SERS, as an analytical tool, has been used in biological applications such as immunoassay, cellular studies, and bacterial detection etc. For achieving significant information from bacterial cell using SERS studies, a colloidal solution of gold (Au) or silver (Ag) nanoparticles (NPs) is required [7].

Commonly, to capture, separate and identify a bacterium from a contaminated sample immunosensor is one of the choices. A typical immunosensor is usually fabricated by immobilizing the specific antibody on the surface of an inert solid support via chemical or physical mechanisms. The iron oxides (Fe$_3$O$_4$ and γ-Fe$_2$O$_3$; IO) NPs, possess their unique magnetic property – superparamagnetism, which enables their stability and dispersion after removing the magnetic. Hence, silica coated IO or magnetic NPs (SMNPs) not only offer improved stability but also help to bind the various chemical and biological molecules covalently at the surface of NPs [8].

In our previous report we have shown the synthesis and application of colloidal Ag NPs and Ag cluster over silica NPs for antibacterial activity [9]. Further, in order to identify the different biomolecules upon the action of Ag NPs on bacterial cells and mechanism of action, here we synthesized and characterized SMNPs. The surface hydroxyl groups of these NPs were activated for covalently immobilization of *E. coli* antibody. These immunomagnetic (IM) NPs were used to capture magnetic separation and concentrate *E. coli* from phosphate buffer saline (PBS) suspension. The captured *E. coli* were treated with colloidal Ag NPs and analyzed using SERS at 1.5 and 2.5 hr of incubation to identify different biomolecules of *E. coli*.

2 EXPERIMENTAL

2.1 Reagents

All the chemicals and reagents were purchased from Sigma Aldrich, (St. Louis, MO, USA) and Fisher Scientific (New Jersey, USA). Polyclonal general goat anti-*E. coli* antibody was purchased from Viro Stat Inc. (Portland, ME, USA).
General strains of environmental *E. coli* were isolated from local fresh water streams; *E. coli* strain ATCC 25922 general strains were provided by Dr. Gregory Bohach (MMBB Department, Univ. of Idaho (UI), Moscow, Idaho, USA. The 1x phosphate buffered saline (PBS) was prepared from a 10x autoclaved stock PBS (100 mM phosphate buffer, 1.37M NaCl, 27 mM KCl, pH 7.2. Deionized (DI; 18.2 MΩ) water was collected from Labconco, water Pro PS.

2.2 Instrumentation
JEOL 1200EX II model was used for transmission electron microscopic (TEM) images and Supera Gemini 35 VP FE-SEM (Zeiss) coupled with Thermoelectron (Zeiss) model was used for Field emission scanning electron microscopic (FE-SEM) imaging. The functional group of the nanoparticles was characterized using Fourier transform-infrared (FT-IR) and Ultraviolet visible (UV-vis) spectrophotometer (model; PharmaSpec UV-1700). Bacterial growth was monitored by plating on agar plates. Visible Raman and SERS spectra were recorded using a Raman analyzer WITec alpha300 Raman Microscopy System (WITec GmbH, Ulm, Germany) equipped with 532 nm and 785 nm excitations. The laser power was used in the range of 0.04 - 2 mW, and the exposure time was 0.5 to 3 sec for comparative purposes.

2.3 Synthesis of Iron Oxide Nanoparticles
The synthesis of IO NPs was carried out using previously reported method [10] and the brown color mixtures of iron oxide were analyzed for magnetite property and through TEM imaging, which gave needle shape structures.

2.4 General Method for SMNPs
50 mL of ethylalcohol (EtOH) 200 proof, 1 mL of DI water and 0.56 mmol tetraethylorthosilicate (TEOS) respectively were mixed into 125 mL round bottom flask and stirred for 30 min. 3.4 mL of ammonium hydroxide (28-30%) solution was added to above solution and stirred at 3000 rpm for 20 min. at RT. The IO NPs (25 mg) suspension in 2 mL of DIW and EtOH (v/v; 1:1) was added drop wise with vigorous stirring over the period of 30 min. The reaction was allowed to stir for 16 h at RT. The brown suspension was separated through magnet and washed with 80% EtOH three times and then SMNPs were cured in vacuum oven at 110 °C for 24 h.

2.5 Determination of Magnetic Property
Magnetic properties of IO and SMNPs samples were measured by placing the dry and weighted powdered sample (~ 10 mg) in vibrating sample magnetometer (VSM; DMS model 1660) at RT. Magnetic hysteresis curve (saturation magnetization vs coercivity) for each samples was recorder by applying magnetic field of -13500 Oe to 13500 Oe.

2.6 Conjugation NPs with Antibody
The 5 mg of SMNPs were treated with cyanogens bromide (CNBr) to activate for covalent coupling with antibody and the coupling with polyclonal goat anti-*E. coli* antibody with activated SMNPs was performed as previously reported method by us [11].

2.7 *E. coli* Capture
The IM NPs (25 µg/mL) were mixed in bacterial suspension from its different dilutions (10^{-6} to 10^{-9}) containing ~1.8 x 10^{4} to ~18 cfu/mL and set up on cyclic rotator for 30 min at rt. Then the NPs were magnetically separated from the bacterial suspension and washed twice with PBS containing 0.1% Tween 20 (PBST). A small aliquot (50 µL) of the washed NPs with bacteria were placed on a sterile LB agar plate and incubated for 12 hr at 37 °C to confirm the presence of bacteria. The remaining aliquots of the samples were used for other analyses.

2.8 Raman Identification
Aqueous suspension (0.2 - 0.5 µL) of sample was placed on glass slide and dried at RT before taking the Raman spectra. The laser beam was focused on a 2 µm area of each sample to image and acquire the Raman spectra. The microscope was used to localize the bacteria. Each spectrum took ~ 5 min to acquire. The performance of the 532 nm lasers was found to be superior for bacterial SERS study over 785 nm laser (data not shown here) in the presence of Ag NPs. For assessing the reproducibility of this approach, three replicate spectra were obtained from each sample from different points of the matrix were taken, focusing on the different spots.

3. Results and Discussion

3.1 SMNPs Synthesis and Characterization
Figure 1a shows the TEM image of irregularly shaped IO NPs of 15±4 nm of size but needle like structures with length of 90±10 nm and width of 4-6 nm are visible. This TEM image is overlapping of NPs gives approx. 90% smaller particles and around 10% needle like nanorods of IO NPs. These IO NPs were treated with silica precursor TEOS results in 75±10 nm SMNPs as shown in Figure 1b. In this image the center black dots are IO NPs and surrounding gray color is comprised silica coating. The physical appearance of these SMNPs is brown color.

VSM (DMS, 1660) magnetometer was used for the quantitative magnetization measurements of IO and SMNPs as shown in Figure 2a. The saturation magnetization (Ms, emu/g) and coercivity (Hc, OE) obtained from these magnetic hysteresis loops (black curve) could be seen for the IO NPs with Ms = 45.17 emu/g and Hc = 9.81 Oe.
Due to the weight increased by non-magnetic silica, the Ms of SMNPS were decreased by increasing the amount of silica used in the synthesis process. Although SIO NPs synthesized here are not superparamagnetism (Ms = 15.08 emu/g and Hc = 13.38 Oe) due to their Hc were above 10 Oe, but they possessed significant saturation magnetization. The visual identification of magnetic property was characterized by applying external magnet outside of vial containing a suspension of SMNPs. The Figure 2b (left vial) shows the suspended SMNPs (0.5 mg/ml in PBS buffer (6.8 pH) and these SMNPs were pulled from suspension through magnet Figure 2b (right vial).

3.2 Capture of E. coli
The 10^-6 to 10^-9 dilutions of E. coli was mixed with IM NPs but the sample containing bacterial cells from 10^-8 dilution (containing ~ 180 cfu/mL) was selected for further study. To confirm the capture of bacteria using IMNPs were mixed with E. coli cell suspension and incubated for 30 min. at RT. After washing NPs were placed on agar plate to incubate further 24 hr.

The agar plate Figure 3a shows incubated cfu/mL of E. coli from 10^-8 dilution suspension, this was used to treat the NPs. While Figure 3b and c, shows plat containing IONPs and SMNPs respectively treated with E. coli cells. These two plates were used as a negative control for the sample, because they should not capture any bacterium. Since, they were not linked with E. coli antibody. The sample plated is shown in Figure 3d. The presence of E. coli cfu on this plate, confirm that the IM NPs are capturing the E. coli cells from its 10^-8 dilution suspension and it is due to antibody binding with the bacterial cell wall receptors.

3.3 SERS Study
The captured E. coli and IM NPs complexes, which were incubated with Ag NPs ~5 h, also monitored using FE-SEM images. Figure 4a,b show different regions of dead and damaged E. coli cell sample, embedded with IMNPs and Ag NPs aggregates. These images clearly show the toxic effect of Ag NPs and therefore act as antibacterial nanomaterial.

The SERS of IM NPs showed characteristic bands of proteins (antibody). A sharp peak for O-Ag bond vibration and interactions of ionic species, adsorbed onto Ag NPs can be seen at 241 cm^-1 [12]. Bands at 560 and 787 cm^-1 are showing Si-O-Si linkage and Fe_2O_3 (maghemite) units of
NPs. The characteristic SERS band for protein comes from phenyl vibration (around 1001 cm\(^{-1}\)), amide I, II and III vibrations (from 1100 to 1600 cm\(^{-1}\)) [13], these bands at 1005, 1096, 1322, and 1598 cm\(^{-1}\) can be seen in Figure 5a and are indicative of antibody attachment with SMNPs.

The SERS spectra of \(\text{E. coli}\) at 1.5 h of incubation with Ag NPs shown in Figure 5b. It shows the major SERS signals for protein (1250 – 1370 cm\(^{-1}\)), lipids (1420 – 1550 cm\(^{-1}\)) and some nucleic acids (1550 – 1670 cm\(^{-1}\)) bands of bacteria. Some of the recent reports have well explained the SERS finger printing of these biomolecules from \(\text{E. coli}\) species [14,15], similar bands were observed in Figure 5b.

The weak band at 581 and 1030 cm\(^{-1}\) corresponds to carbohydrate, mainly \(-\text{C-C-}, \text{C-O, and C-O-H}\) skeleton. The weak bands at 625 cm\(^{-1}\) for COO- group of protein peptides and aminoacids. 1105 cm\(^{-1}\) band is from glass slide. 1195, 1265, and 1323 cm\(^{-1}\) bands are showing amide I and III for N-H, alkane CH\(_2\) twist, rock mode, and C-H linkage respectively. The \(\delta (\text{CH}_{2})\) from lipid group can be seen at 1454 cm\(^{-1}\). Adenine and guanine band appears at around 1485 cm\(^{-1}\). The band at about 1548 cm\(^{-1}\) comes from C=C (lipid), \(\delta \) (N-H) and \(\nu\) (C-N) from amide II group.

**FIGURE 5.** SERS spectra in presence of colloidal Ag (a) anti-\(\text{E. coli}\) antibody functionalized SMNPs and (b) captured \(\text{E. coli}\) using antibody-SMNPs.

3 CONCLUSION

In this paper, we demonstrate the synthesis of SMNPs. These SMNPs were activated using cyanogens bromide and covalently linked with polyclonal \(\text{E. coli}\) antibody. The immuno SMNPs were used to capture and isolate \(\text{E. coli}\) from 180 cfu/mL aqueous suspension and treated with colloidal Ag NPs. The SERS spectra and SEM images shows the identification of various biomolecules of \(\text{E. coli}\) cell. The immune-magnetic isolation of bacterial cells for capture, concentration and SERS spectral analysis is a quick and reliable method for identification of bacterial species without extensive experiments and statistical data treatment.

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