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Fluorimetric detection of pathogenic bacteria using magnetic carbon dots

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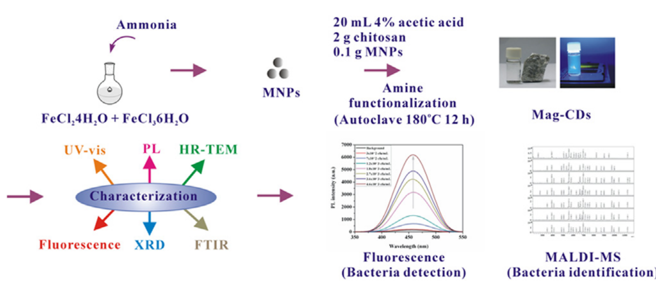
HIGHLIGHTS

- Magnetic carbon dots with amine functionalization was newly proposed.
- Magnetic separation and fluorometric detection of pathogenic bacteria achieved.
- Achieved very low limit of detection of pathogenic bacteria using this technique.

GRAPHICAL ABSTRACT



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ABSTRACT

A novel and facile approach of pathogenic bacteria detection, which utilizes fluorescent sensing and bacteria capture with Magnetic carbon dots (Mag-CDs), was proposed in this work. Magnetic nanoparticles were synthesized and then decorated with C-dots, and further functionalized with amine groups (chitosan). In this way, bacteria were strongly anchored on the hybrid material Mag-CDs for highly sensitive fluorescent detection. The Mag-CDs were characterized by UV–vis, FT-IR spectra, TEM images, XRD, and EDX. The characterizations validate the fabrication of amine-Mag-CDs and the promising applications of this material. Fluorescence spectroscope and MALDI-MS were used for the detection and identification of bacterial strains, respectively. The limit of detection for *Staphylococcus aureus* and *Escherichia coli* was found to be 3×10^2 and 3.5×10^2 cfu mL^{-1}, respectively. With these encouraging results, it is expected that it would open revenues for promising applications of Mag-CDs nanomaterial.

1. Introduction

Pathogenic bacterial detection and quantification have been pursued in the long term and are extremely important in biological, food related items, and environmental samples [1]. Especially *Escherichia coli* and *Staphylococcus aureus* are opportunistic

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bacterial species leading to human infections; they are majorly found in urinary tract infections. The unique nano-based magnetic nanoparticles applied on bio-sensing methodology [2,3] prompts us to apply them extendedly. In recent years, carbon nanomaterials have received considerable attention primarily because of their unique properties. Among various carbon nanomaterials like graphene sheet, graphene oxide, and carbon nanotube, carbon dots have attracted attention nowadays [4]. Carbon dots were accidentally discovered, which have unique properties such as, strong photoluminescence, wavelength-dependent emission, relative stability, attractive prospects for bioimaging, high photostability, tunable excitation and emission wavelength, exceptional biocompatibility and eco-friendliness [5–7], etc. Even presented in the combined form C-dots also have variety of potential applications with CaO, ZnO, TiO_2, and Al_2O_3 [8], and so forth. Recently, much research interest has been extended to hybrid nanomaterials in biology, physics, engineering and chemistry fields. Carbon-coated nanomaterials have been extensively used for magnetic resonance imaging study, and carbon dots can also be passivated with amine terminated compounds through amine functionalization [9,10].

The hybrid nanomaterials of carbon dots coated magnetic nanoparticles combine both fluorescent and magnetic properties in one material. The particular functionalization of amine group is originated or sourced from precursor chitosan [11]. One of the advantages of carbon dots lie behind its feature of providing better dispersibility, stability to iron oxide nanoparticles compare to baer iron oxide nanoparticles. Additionally, C-dots coated iron oxide nanoparticles are prone to prevent the oxidation and corrosion of iron oxide nanoparticles [8]. Fe_3O_4/carbon synthesis was first proposed by Zhifei research group [12], the important application of iron oxide is iron oxide/gold nanoparticles, which is particularly of interest to the controlled magnetic field [13]. In general, the synthesis of carbon-coated materials have been proposed including electric arc discharge, catalytic pyrolysis of organic compounds, and the hydrothermal method [14]. Carbon/Fe_3O_4 nanoparticles have been specifically applied as sorbents in solid-phase extraction (SPE) from environmental water samples, because the carbon dots have been proved for high capacity adsorption and extraction efficiency due to large surface area to small volume ratios [14]. To date, carbon dots decorated on magnetic nanoparticles has not yet been studied. Furthermore, the fluorescent property of carbon dots and the amine-functionalized nature make them applicable for trapping or as probes for pathogenic bacteria by using Mag-CDs.

Since last decade, research groups [15] such as; Arruda et al., 2009 [16]; Gao et al., 2009 [17]; Lu et al., 2010 [18]; Sanvicens et al., 2009 [19], have been engaged in development of efficient and cost-effective techniques based on magnetic nanoparticles for pathogen detection. Label-free magnetic nanoparticle-antibody conjugates were prepared for *E. coli* [20,21]. Detection of bacteria using carbohydrate-based fluorescent polymer [22–25], nanoparticle mediated [26–30], fluorescent detection [31,32], colorimetric bacterial sensing [33] have also been proposed. Specifically, marine pathogen detected by vancomycin-coated magnetic nanoparticles was proposed by Wan et al., 2014 [34]. Further development in detection and identification of bacteria by quaternized magnetic nanoparticle-fluorescent polymer system [34] is also known. Usually, magnetic nanoparticles were engineered with conjugation, tagging, chemical agent, and antibody applications [35]. Chitosan coated iron nanoparticles were synthesized and applied as environmental trace pollutants extraction [36–38] and drug sensors [39]. In our currently synthesized method, no tagging or use of polymer is needed and it provides many advantages such as fluorescent and magnetic properties by the straightforward and cost-effective approach for highly preconcentration capability for capture and sensitive detection for pathogenic bacteria. Instead of applying amine functionalization on iron oxide nanoparticles for pathogenic bacterial removal [40], this easy and feasible method uses the readily accessible precursor chitosan [41] and synthesizes in situ.

Previous reports have described the combination of carbon coated iron oxide nanoparticles used in magnetic resonance imaging [8]. We tried to synthesize the Mag-CDs and applied for detection of pathogenic bacteria using advanced fluorescence and MALDI-MS techniques. We demonstrated the synthesis and use of amine functionalized Mag-CDs as highly sensitive affinity probes for bacterial detection for the first time, utilizing the magnetic property of Mag-CDs for collecting and amine functionalized carbon dots for extracting pathogenic bacteria.

2. Experimental section

2.1. Materials and method

$FeCl_2 \cdot 4H_2O$ was obtained from Alfa Aesar, Johnson Matthey Company (USA). $FeCl_3 \cdot 6H_2O$ was from Showa Chemical Co. LTD (Japan). Ammonia solution (28%) was purchased from Fluka (Steinheim, Germany). Acetic acid purchased from Sigma–Aldrich (USA). Chitosan (low molecular weight; 50,000–190,000 g mol^{-1}, 75–85% deacetylation) and Trifluoroacetic acid (TFA) were purchased from Sigma–Aldrich (Germany). Sinapic acid (SA) was obtained from Alfa Aesar (Ward Hill, MA 01835, USA). Ethanol and acetonitrile (ACN) were obtained from J. T. Baker (Phillipsburg, NJ, USA). Ultrapure water from a Milli-Q Plus water purification system (18.2 MΩ·cm, Millipore, and Bedford, MA, USA) was used for all experiments. All reagents used in the preparation process were of analytical reagent grade and were used without further purification.

Bacterial Source and cultivation A Biosafety, level-1 cabinet (Nuaire, Plymouth, MN, USA) was used for all bacteria experiments. The standard bacterial strains *S. aureus* (BCRC 10451); *E. coli* (BCRC 12570) were purchased from the culture collection at Bio resource Collection and Research Center (BCRC), Hsin-Chu, Taiwan. Both bacteria were cultured on Luria–Bertani (LB.) agar plates (Bio Basic Inc. SD 7002 amended with 15 g L^{-1} agar) for 24 h incubation at 37 °C. The bacteria cells were transferred from Luria–Bertani agar plate to 1 mL PBS buffer solution (pH = 7.2) and used for further analysis. Bacterial concentration in the suspension was estimated by the traditional plate counting method. All the glassware and media used for the studies were autoclaved at 15 lbs. (pressure) for 45 min.

2.2. Instrumental utility

The structure and morphology of as-synthesized amine functionalized Mag-CDs were characterized by X-ray diffraction (XRD; Phillip, The Netherlands) and transmission electron microscopy (TEM, Phillip CM200, Switzerland). The Fourier transform infrared (FT-IR) spectra of Mag-CDs and iron oxide nanoparticles were obtained on a FT-IR spectrometer (Spectrum 100, Perkin Elmer, USA). The Energy-dispersive X-ray spectrometer (EDX) (JOEL 6700F, Japan) was used for the elemental detection of Mag-CDs.

The excitations as well as the emission spectra were recorded using the fluorescence spectroscope (Hitachi F2700, Japan). A UV–vis. spectroscope (Thermo, Evolution 201, USA) was used to study the spectral properties of Mag-CDs. MALDI-TOF-MS analyses were performed employing delayed extraction in positive ion mode on a time-of-flight mass spectrometer (Microflex, Bruker Daltonics, Bremen, Germany) with a 1.25 m flight tube. Desorption and