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Dopamine coated Fe₃O₄ nanoparticles as enzyme mimics for the sensitive detection of bacteria†

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Abstract

We report a simple and economical colorimetric bacterial sensing strategy with catalytic amplification using dopamine-capped iron oxide (Dop-Fe₃O₄) nanoparticles. These nanoparticles catalyse the oxidation of a chromogenic substrate in the presence of H₂O₂ into a green colored product. The catalytic activity of the nanoparticles is inhibited in the presence of bacteria, providing naked eye detection of bacteria at 10⁴ cfu/mL and by spectrophotometric detection down to 10² cfu/mL.

Graphical Abstract

Colorimetric sensing of bacteria using dopamine-functionalized iron oxide nanoparticles.

According to the World Health Organization, more than 25% of all global deaths (15 million out of total 57 million) are due to infectious diseases, many of which are caused by bacteria.¹ Major sources of infectious diseases are contaminated water and food.² Microbial detection is a key step in the prevention of these types of infectious and deadly diseases.³ The standard technique for bacteria determination is culturing and plating, a relatively inexpensive and sensitive technique, but it requires lengthy incubation for reliable results.⁴ Molecularly-amplified methods such as PCR, NASBA (nucleic acid sequence based amplification) and enzyme amplified methods (e.g. ELISA) have reduced detection time and

†Electronic Supplementary Information (ESI) available. Experimental details, characterization, as well as additional figures for sensing strategy. See DOI: 10.1039/x0xx00000x

enhanced overall sensitivity, but these techniques are expensive and require laboratory facilities.⁵ Recently, nanomaterials (carbon nanotubes, silicon nanowires, magnetic nanoparticles, silver nanoparticles, gold nanoparticles/nanorods, polymer brushes etc.) have been integrated into sensing strategies to help improve detection and discrimination of bacteria.^{6–13}

Enzyme-amplified colorimetric sensing methods are attractive because they provide visual readouts without the need of sophisticated instrumentation.¹¹ However, these methods have issues due to the high cost of enzymes, their instability, and challenges in extraction and purification.^{14,15} Synthetic enzymatic mimics have been used to address these concerns, including metal and metal oxide/sulphide nanoparticles (Au, Ag, Pt, Au@Pt, Pd-Ir, Fe₃O₄, CeO₂, V₂O₅, CuO, MnO₂, CoFeO₄, Fe₃S₄, CuS and CoS), carbon nanotubes etc., feature higher stability than enzymes over a wide range of pH levels and temperature.^{16–32} More widely, the enzymatic mimicking property of Fe₃O₄-NPs has been used to replace horseradish peroxidase (HRP) in traditional immunoassay techniques and to achieve colorimetric sensing of proteins using catalytically amplified sensor arrays.^{21, 33} Inspired by this catalytically amplified Fe₃O₄-NPs based colorimetric sensing of proteins,³⁴ we report the rapid enzyme-free colorimetric detection of bacteria by using dopamine-capped magnetic ferrite nanoparticles (Dop-Fe₃O₄-NPs) that serve as both recognition and transduction elements. As shown in Fig. 1, a colorimetric response is produced by the oxidation of a chromogenic substrate 2, 2'-azino- bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) into a green colored product by a peroxidase mimetic nanoparticles (Dop-Fe₃O₄-NPs) in the presence of hydrogen peroxide (H₂O₂). Binding of the cationic nanoparticles³⁴ to the anionic surface of bacteria blocks access of substrate to the nanoparticle core, providing turn-off colorimetric sensing of bacteria.

Dop-Fe₃O₄ NPs were prepared by modifying Haiou's method for bacterial sensing studies.³⁵ Briefly, diethylene glycol (DEG) capped iron oxide nanoparticles were synthesized by co-precipitation at 220 °C followed by post-functionalization with dopamine through labile ligand exchange method to avoid polydopamine formation in basic media (Fig. S1). The transmission electron microscopy (TEM) image indicates narrow size distribution of Dop-Fe₃O₄ NPs with an average size of ca. 8 nm (Fig. 2A). Powder XRD spectroscopy was used to describe the phase characteristics of Fe₃O₄ NPs (Fig. S3) whereas EDX spectroscopy was performed for elemental analysis (Fig. S4). The high dispersability of nanoparticles in aqueous media along with a zeta potential value of +31.3 mV indicates the surface modification with positively charged dopamine ligands through catechol-like moiety, which was further confirmed by the prominent peaks appearing at 1612 cm⁻¹ and 1502 cm⁻¹ due to free N—H primary amino group bending vibrations by Fourier transform infrared (FT-IR) spectroscopy (Fig. 2B) and the fragmentation pattern observed at m/z values of 151, 134 and 120 by MALDI-TOF spectrometry (Fig. 2C and S5).

For catalytic activity studies, a colorimetric reaction of ABTS with H₂O₂ was performed. The colorimetric response of oxidized ABTS with H₂O₂ was amplified in the presence of Dop-Fe₃O₄ nanoparticles as compared to the reaction in the absence of nanoparticles (Fig. 3). Furthermore this response was completely turned off in the presence of high concentration of bacteria (i.e. 10⁸ cfu/mL). The blockage in catalytic activity of

nanoparticles resulted from electrostatic interaction with anionic cell surface of bacteria,⁹ indicating that this catalytically amplified colorimetric strategy can be used for sensing of bacteria.

We used *Escherichia coli* (XL1), a Gram-negative bacteria, and *Bacillus subtilis* (DH_α), a Gram-positive bacteria, as model analytes for bacterial sensing studies. A turn-off colorimetric strategy was used for the detection of bacteria. The intensity of colorimetric response due to catalytic activity of Dop-Fe₃O₄ nanoparticles was decreased by the addition of different concentrations of *E. coli* and eventually was completely inhibited in the presence of high concentration of bacteria i.e., 10⁸ cfu/mL, resulting in detectable visible color change down to 10⁴ cfu/mL (Fig. 4A). The gradual decrease in colorimetric response was also monitored by recording the absorption spectra of oxidized ABTS in the presence of a range of *E.coli* concentrations from 10² to 10⁸ cfu/mL (Fig. S8). The gradual decrease in optical density measurements at 420nm in the presence of bacteria further improved the limit of detection (LOD) down to 10² cfu/mL (Fig. 4B).

The changes in catalytic activity of Dop-Fe₃O₄ nanoparticles were also observed by using the Gram-positive model analyte *Bacillus subtilis* (DH_α). We observed a detectable visual colorimetric change down to 10⁴ cells/mL of *Bacillus subtilis* (*B.sub*) concentration. When optical density measurements at 420 nm of the colorimetric reaction were recorded in the presence of *B.sub* (DH_α), the observed results were comparable to *E.coli* (Fig. S9). Overall, this turn-off colorimetric sensor provides naked eye limit of detection of bacteria for *E.coli* and *B.sub* down to 10⁴ cfu/mL, and further down to 10² cfu/mL by using spectroscopic mode of multiplate reader.

In conclusion, a simple, facile and an inexpensive enzyme free turn-off colorimetric methodology is developed by integration of Dop-Fe₃O₄ nanoparticles. These nanoparticles act as both transducer and recognition element by interacting with the surface of bacteria, which results in naked eye detection of bacterial concentration as low as 10⁴ cfu/mL in solution, which is further improved to 10² cfu/mL by spectrophotometric measurement. This sensing methodology provides a low-cost strategy for determination of drinking water contamination that can be further used for microfluidic/paper devices for biosensing and biomedical diagnostics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Catalytically amplified colorimetric sensing of bacteria by using Dop-Fe₃O₄ NPs as enzymatic mimetic.

Figure 2.
(A) TEM image of Dop- Fe₃O₄ NPs; (B) FTIR-spectra of a: dopamine ligand and b: Dop- Fe₃O₄ NPs; (C) MALDI-TOF spectra showing fragmentation pattern of Dop- Fe₃O₄ NPs.

Figure 3.

Optical density measurements (OD) when ABTS reacts with H_2O_2 (A) in the absence of $\text{Dop-Fe}_3\text{O}_4$ NPs, (B) in the presence of $\text{Dop-Fe}_3\text{O}_4$ NPs, and (C) in the presence of $\text{Dop-Fe}_3\text{O}_4$ NPs and high concentration (10^8 cfu/mL) of bacteria respectively. Inset showing the visual colorimetric response. OD at 420 nm, 0.67 mM ABTS, 6.25 mM hydrogen peroxide and 20 $\mu\text{g/mL}$ $\text{Dop-Fe}_3\text{O}_4$ NPs (Fe 0.367 ppm solution) in 5 mM sodium acetate buffer (pH 4.6).

Figure 4.

(A) Colorimetric response when ABTS is reacting with H₂O₂ catalysed by Dop-Fe₃O₄ NPs in the presence of different concentrations of bacteria; (B) Graph of sensor response, with line graph inset showing gradual decrease in optical density at 420 nm with increasing bacterial count.