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Magnetic Glyco-nanoparticles: A Unique Tool for Rapid Pathogen Detection, Decontamination, and Strain Differentiation

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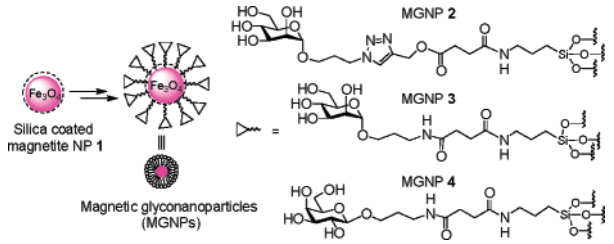
The appealing mechanisms by which nanocomposites knit biomolecules not only lend credence in designing novel nanosensors but considerably advance medical applications.[1] Recent headline news about *Escherichia coli* (*E. coli*) contamination in produce and *Bacillus anthracis* attacks pinpoint the urgent need for an effective method for microbial decontamination and rapid detection without time-consuming cell culturing. It is known that many bacteria use mammalian cell surface carbohydrates as anchors for attachments, which subsequently results in infection.[2] The unique combination of magnetic nanocomposites and diverse carbohydrate bioactivities prompts us to embark on a biosensing research program. Herein, we report a magnetic glyco-nanoparticle (MGNP)-based system to not only detect *E. coli* within 5 min, but also remove up to 88% of the target bacteria from the medium. Furthermore, the identities of three different *E. coli* strains were easily determined on the basis of the response patterns to two MGNPs highlighting their potential in biosensing.

It is advantageous to use magnetic nanoparticles (NPs) for detection. The high surface/volume ratio offers more contact surface area for attaching carbohydrates and for capturing pathogens.[3] The sizes of NPs are typically about 2 orders of magnitude smaller than a bacterium, which allows the attachment of multiple NPs onto a bacterial cell rendering easy magnet-mediated separation.[3,4] Moreover, the small NPs have faster kinetics in solution as compared to their micrometer-sized counterparts, which can result in fast detection.

Our journey commenced with functionalization of silica-coated magnetite NP (NP **1**) with D-mannose (Man) through either a triazole linker (MGNP **2**) formed by the [2+3] Huisgen reaction[5] or an amide linkage (MGNP **3**) (Schemes 1 and S1). With our covalent approach,[6] all carbohydrates are uniformly oriented on the NP surface, which is crucial for high performances in cell-capturing studies.[7] All MGNPs were characterized by X-ray diffraction, transmission electron microscopy (TEM), thermogravimetric analyses, and IR spectroscopy (Figures S1−4).

To ensure that carbohydrates on MGNP retain their binding abilities, the interaction between various MGNPs with a mannose binding lectin, concanavalin A (Con A),[8] was first investigated. Carbohydrate−lectin interaction[9] is central in devising our biosensor. After mixing NPs with fluorescein-labeled Con A, a magnetic field was applied to the mixture through a handheld magnet inducing aggregation of magnetic NPs on the side of the vial. The residual fluorescence of supernatants was then recorded (Figure S5). With MGNP **3**, the emission intensity of the supernatant decreased 87% indicating that most Con A was removed by **3**. Triazole linked MGNP **2** was less efficient accounting for a 60% emission decrease probably because of the low efficiency of the

Scheme 1



Huisgen reaction with immobilized alkynes.[5] NP **1** without carbohydrates (control) did not remove any Con A, proving that the separation of Con A is due to its interaction with carbohydrates, rather than the nonspecific absorption to NP surface. Incubation of 100 mM mannose with MGNP **3** (2 mg/mL) and Con A did not increase the intensity of residual emission of the supernatant after magnetic separation, suggesting that the avidity of MGNP **3** to Con A is at least 200-fold higher than the affinity of the monomeric mannose. These results reveal the multivalent appeal of MGNP with ligand clustering leading to strong binding.

The above experiments set the stage for detection of bacterium *E. coli*. After incubating MGNP **3** (2 mg/mL) with solutions of an *E. coli* strain ORN178 (10^3−10^7 cells/mL in PBS buffer) for a few minutes, a magnetic field was applied separating MGNP/*E. coli* aggregates (Figure 1). The supernatants were carefully removed and the remaining aggregates were washed thoroughly, stained with a fluoresecent dye (PicoGreen), transferred to a glass slide, and imaged. Fluorescent microscopic imaging showed that *E. coli* can be reliably detected with a limit of 10^4 cells/mL (Figure 2a). With NP **1**, no bacteria were observed on the slides.

Following the same protocol, we enumerated bacteria in the aggregate and supernatant using the fluorescent microscope. The capture efficiency was calculated by dividing the number of *E. coli* in MGNP aggregate over the total number of cells in both the supernatant and the aggregate. High capture efficiencies up to 88% can be achieved with 45 min incubation (Table S1). Next, the effect of incubation time was examined. Interestingly, even in just 5 min, *E. coli* can be detected with a high capture efficiency of 65% (Table S2). MGNP/*E. coli* complexes were then imaged by TEM with MGNP aggregates observed on the surface, at the lateral ends and

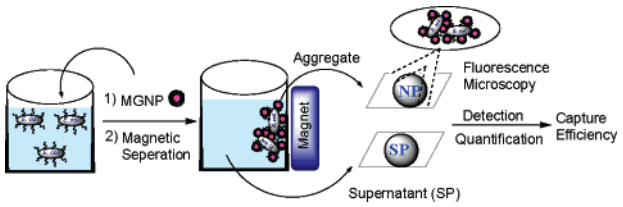


Figure 1. Schematic demonstration of pathogen detection by MGNPs.

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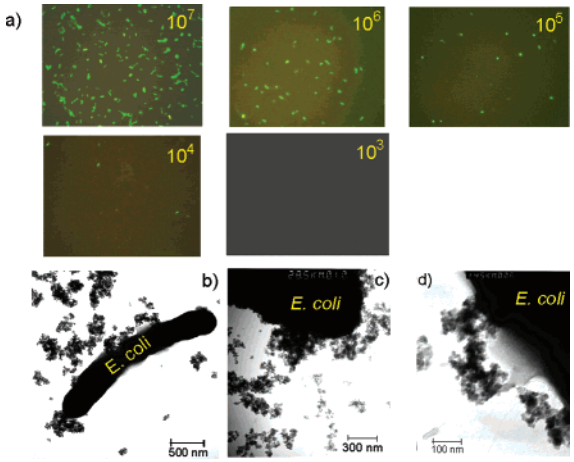

Figure 2. (a) Representative fluorescence microscopic images of captured *E. coli*. The concentration (cells/mL) of bacteria incubated with MGNP **3** is indicated on each image (see Supporting Information for experimental details). (b−d) TEM images of MGNP **3**/*E. coli* complexes.

along the pili[10] of *E. coli* cells (Figure 2b−d, Figure S4). To the best of our knowledge, although glyco-nanoparticles have been studied as bioprobes for pathogens,[11] this is the first time that MGNPs have been used for bacterium detection and decontamination.[12,13]

The capture efficiency using MGNP **3** is much higher than the 10∼30% range typically observed with antibody or lectin functionalized magnetic particles,[14,15] which can be difficult to fabricate because of challenges in immobilizing biomacromolecules.[15] Furthermore, the orientation of the antibody/lectin on a NP surface is difficult to control, which may affect their binding capacities.[16]

To further demonstrate the advantages of MGNPs, we explore the possibility of bacterium differentiation. It is known that several bacteria may bind with the same carbohydrate albeit with various affinities.[17] This provides a unique opportunity to use a MGNP array system, where the selective binding of a microbe to various carbohydrates will lead to different responses. The resulting characteristic response patterns[18] will then allow differentiation of bacteria. As a proof-of-principle, we synthesized galactose (Gal) functionalized MGNP **4** in a similar manner as Man-MGNP **3** and investigated the usage of these two MGNPs to rapidly detect and differentiate three *E. coli* strains: ORN178, ORN208 a mutant strain with greatly reduced mannose binding affinity,[19] and an environmental strain (ES) isolated from Lake Erie with unknown carbohydrate binding specificity. While 65% of ORN178 was captured by MGNP **3**, only 15% was caught by MGNP **4** (Table S3). The mutant strain ORN208 can still be trapped by both MGNPs, although at lower levels. With the ES strain, high capture efficiencies of 70% and 75% were achieved by MGNPs **3** and **4**, respectively, suggesting its strong binding with both mannose and galactose.

The response patterns of the three *E. coli* strains to Man-MGNP **3** and Gal-MGNP **4** allow us to easily determine the microbial identity: ORN178 (Man strong, Gal weak), ORN208 (Man weak, Gal weak), and ES (Man strong, Gal strong) (Figure 3). The ability to distinguish pathogen strains can have clinical applications since the virulence of many pathogens can be correlated with carbohydrate binding specificity.[20] Moreover, the nondestructive nature of MGNP binding can allow the concentration and recovery of pathogens and further analysis by other techniques.[13,21]

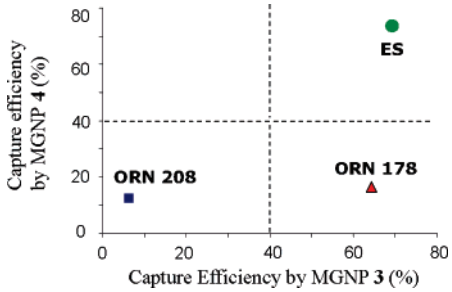

Figure 3. *E. coli* strain differentiation by MGNPs **3** and **4**.

In conclusion, we demonstrate the potential of sugar-coated magnetic nanoparticles for fast bacterial detection and removal, which provides an attractive avenue for pathogen decontamination and diagnostic applications.

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Supporting Information Available: Procedures for MGNP fabrication and characterization; procedures for pathogen detection and differentiation; selected NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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