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Using Biofunctional Magnetic Nanoparticles to Capture Vancomycin-Resistant Enterococci and Other Gram-Positive Bacteria at Ultralow Concentration

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Instant and sensitive detection of pathogens (e.g., bacteria, viruses) at ultralow concentrations without time-consuming procedures, such as incubation or amplification by polymerase chain reaction, offers obvious clinical benefits. However, there is, to date, no general and satisfactory assay that could detect bacteria at concentrations of $<10^2$ cfu/mL without pre-enriching bacteria via a culture process.[1] This communication reports a protocol that uses biofunctional magnetic nanoparticles to capture and detect of vancomycin-resistant enterococci (VRE) and other Gram-positive bacteria at concentrations of $\sim 10^1$ cfu/mL within an hour.

Compared to magnetic beads (1−5 μm in diameter) used in biological separations, magnetic nanoparticles[2,3] (less than 10 nm in diameter) promise high-performance because of their large surface/volume ratios and easy entry into cells.[4] Despite intense efforts in the study of magnetic nanoparticles, the application of magnetic nanoparticles in biomedicine is just emerging.[5] To take advantage of magnetic nanoparticles and ligand−receptor interactions for fast pathogen detection, we designed a system that combines two kinds of interactions: (1) magnetic dipole interactions that aggregate the magnetic nanoparticles under magnetic field and (2) specific multiple ligand−receptor interactions (i.e., polyvalent interactions[6]) that offer high avidity between magnetic nanoparticles and bacteria when the ligands covalently bond to the magnetic nanoparticles. To prove the concept, we attached vancomycin (Van), a broad spectrum antibiotic, to the surface of FePt nanoparticles[3] (3−4 nm in diameter). Van can bind to the terminal peptide, D-Ala-D-Ala, on the cell wall of a Gram-positive bacterium via hydrogen bonds (Scheme 1),[7,8] and previous studies have demonstrated that multivalent Vans have high affinities toward multiple D-Ala-D-Ala receptors.[9] Our designed conjugate of Van and FePt magnetic nanoparticle (FePt−Van, **1**) exhibited high sensitivity to bacteria whose cell walls express D-Ala-D-Ala as the terminal peptides and captured those bacteria at a concentration as low as ~4 cfu/mL. More importantly, we proved that **1** effectively captures VRE, a life-threatening pathogen, at a concentration of 10^1−10^2 cfu/mL via polyvalent interactions.

Scheme 1 displays both the structure of the FePt−Van nanoparticles (**1**) and that of the control, FePt−NH_2 nanoparticles (**2**).[10] Figure 1A shows the general experimental procedure.[11] After mixing the solution of **1** (~11 μg/mL) with a solution of a bacterium for ~10 min, we used a point magnet (with the tip field strength at ~400 G) to capture the "magnetized" bacteria (i.e. bacteria with magnetic nanoparticles bounded to the cell surface) onto the wall of the vial (Figure 2A). After the removal of remaining solution and wash of the aggregates on the wall (with a magnet outside), we transferred the aggregates to a substrate for microscopic analysis.

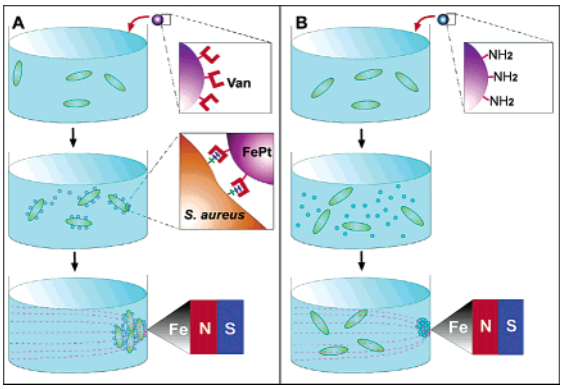
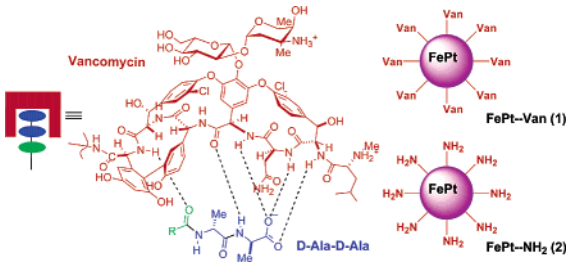


Figure 1. Illustration of the capture of bacteria by vancomycin-conjugated magnetic nanoparticles (A) via a plausible multivalent interaction and the corresponding control experiment (B).

Scheme 1



We repeated the same procedure using FePt nanoparticles capped with amine groups as the control experiment (Figure 1B).

We found that **1** indeed captured Gram-positive strains such as *Staphylococcus aureus* (8 cfu/mL), *S. epidermidis* (10 cfu/mL), and a coagulase negative staphylococci (CNS, 4 cfu/mL). Figure 2A shows the optical image of the isolated *S. aureus* when **1** is used, and the scanning electron micrograph (SEM) confirms that these "magnetized" *S. aureus* aggregate with **1** under magnetic field (Figure 2C). When **2** is used, neither the optical microscopy (Figure 2B) nor the SEM (Figure 2D) shows *S. aureus*, suggesting that **1** binds to *S. aureus* specifically due to the molecular recognition. We also used **1** and **2** to treat solutions containing other Gram-positive strains, such as *S. epidermidis* or CNS. We found that **1** captures both *S. epidermidis* and CNS, but **2** captures neither of them, which further confirms **1**'s binding to the Gram-positive bacteria via specific interaction, similar to the case of *S. aureus*. Panels E and F of Figure 2 show the SEM images of those bacteria and the aggregates of **1**. In addition, when *S. epidermidis* (15 cfu/mL) was mixed with white blood cells (WBC, 100 cfu/mL), **1** only specifically binds to *S. epidermidis*.[11]

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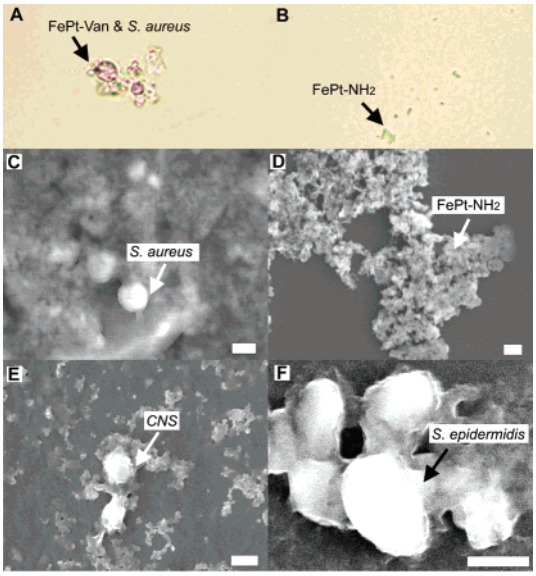

Figure 2. (A, C) Optical image and a SEM image show the aggregates of *S. aureus* and **1**; (B, D) an optical image and a SEM image show the aggregates of **2** after mixing with *S. aureus*; SEM image of the aggregates of (E) *CNS* and **1**; and (F) *S. epidermidis* and **1** (magnification for a and b = 400, scale bars = 1 μm; the exact counts of bacteria were confirmed by back titration[11]).

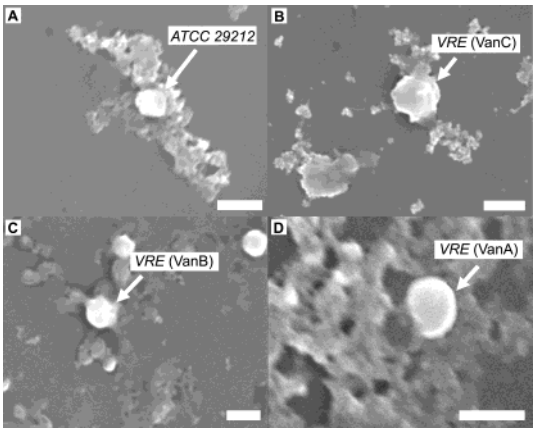

Figure 3. SEM images of (A) *Enteroccocus faecalis* (ATCC 29212, a Van sensitive strain, 26 cfu/mL); (B) *E. gallinarum* (a VanC strain, 84 cfu/mL); (C) *E. faecium* (a VanB strain, 22 cfu/mL); and (D) *E. faecium* (a VanA strain, 34 cfu/mL) and the aggregates of **1** (scale bars = 1 μm, the genotypes of the strains were determined by PCR, and the exact counts of bacteria were confirmed by back titration[11]).

Despite the fact that the affinity of Van to the terminal peptides on the cell wall of VRE decreases due to mutation,[7] our previous study showed that Van-decorated gold nanoparticles[12] bound strongly to VRE. Similarly, in the current experiment, **1** binds to VRE with high avidity via polyvalent interactions and captures VRE. Figure 3 shows the SEM images of VRE or ATCC 29212 (a vancomycin-sensitive strain as a positive control) that is captured by the Van-decorated FePt nanoparticles. Transmission electron microscopy also shows that **1** binds to the surface of the VRE cell.[11] These results not only further demonstrate the effectiveness of **1** as a polyvalent ligand, but also provide a useful method to capture VRE.

In summary, by combining FePt magnetic nanoparticles with a polyvalent ligand−receptor interaction, we have demonstrated a sensitive and quick assay for bacteria. Recently, we also showed that **1** can capture Gram-negative bacteria at a slightly higher concentration (15 cfu/mL).[10] The existing archives of optical and SEM profiles of most bacteria allow easy identification of these captured bacteria. The sensitivity demonstrated in this work is comparable to that of assays based on PCR.[13] The size of the polyvalent nanoparticle (3−4 nm), which is in the same order as antibodies such as IgM (6−8 nm), may also contribute to the high performance of **1**.

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Supporting Information Available: The TEM of FePt, **1**, and captured VRE, details of capture, and the control with WBC (PDF). This material is available free of charge via the Internet at http://pub.acs.org.

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