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(54) **BIOFUNCTIONAL MAGNETIC NANOPARTICLES FOR PATHOGEN DETECTION**

(75) Inventors: **Bing Xu**, Kowloon (HK); **Pak Leung Ho**, Kwai Chung (HK); **Hongwei Gu**, Kowloon (HK)

Correspondence Address:
LAW OFFICES OF ALBERT WAI-KIT CHAN, LLC
WORLD PLAZA, SUITE 604
141-07 20TH AVENUE
WHITESTONE, NY 11357 (US)

(73) Assignees: **The Hong Kong University of Science and Technology; The University of Hong Kong**

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(57) **ABSTRACT**

This invention provides a method of detecting pathogens comprising the steps of: (a) contacting a sufficient amount of biofunctional magnetic nanoparticles with an appropriate sample for an appropriate period of time to permit the formation of complexes between the pathogens in the sample and the nanoparticles; (b) using a magnetic field to aggregate said complexes; and (c) detecting said complexes. The method may further comprise the additional step of removing said complexes. The biofunctional magnetic nanoparticles are preferably a conjugate of vancomycin and FePt. The pathogens may be bacteria or viruses, and the sample may be a solid, liquid, or gas. Detection may involve conventional fluorescence assay, enzyme-linked immunosorbent assay (ELISA), optical microscope, electron microscope, or a combination thereof. The sensitivity of detection for the method is at least as low as 10 colony forming units (cfu) of the pathogens in one milliliter of solution within one hour.

Figure 1

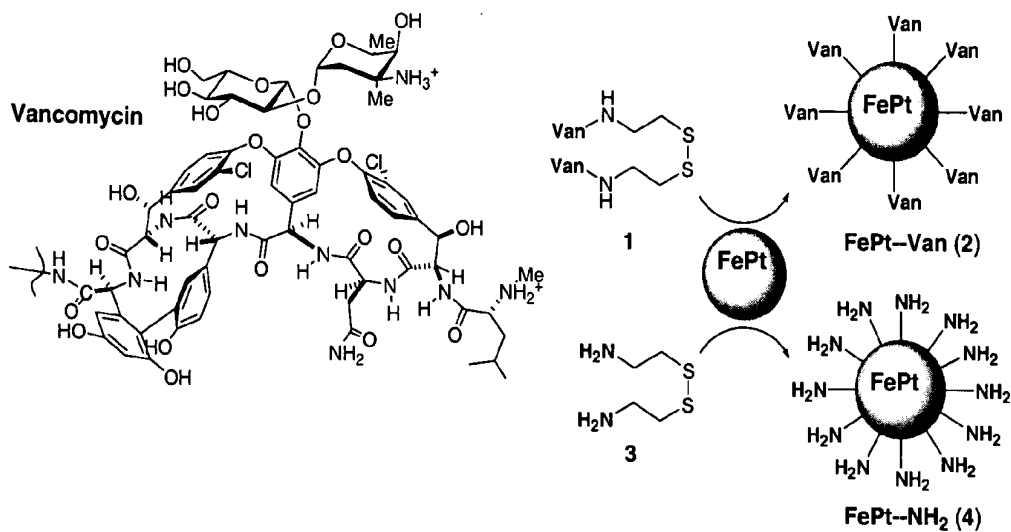


Figure 2

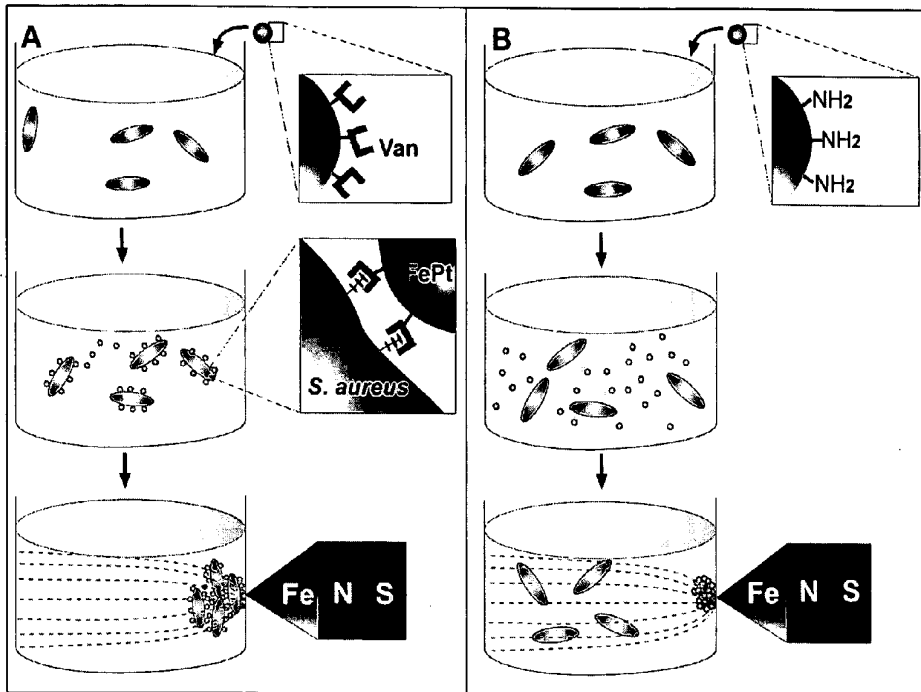


Figure 3

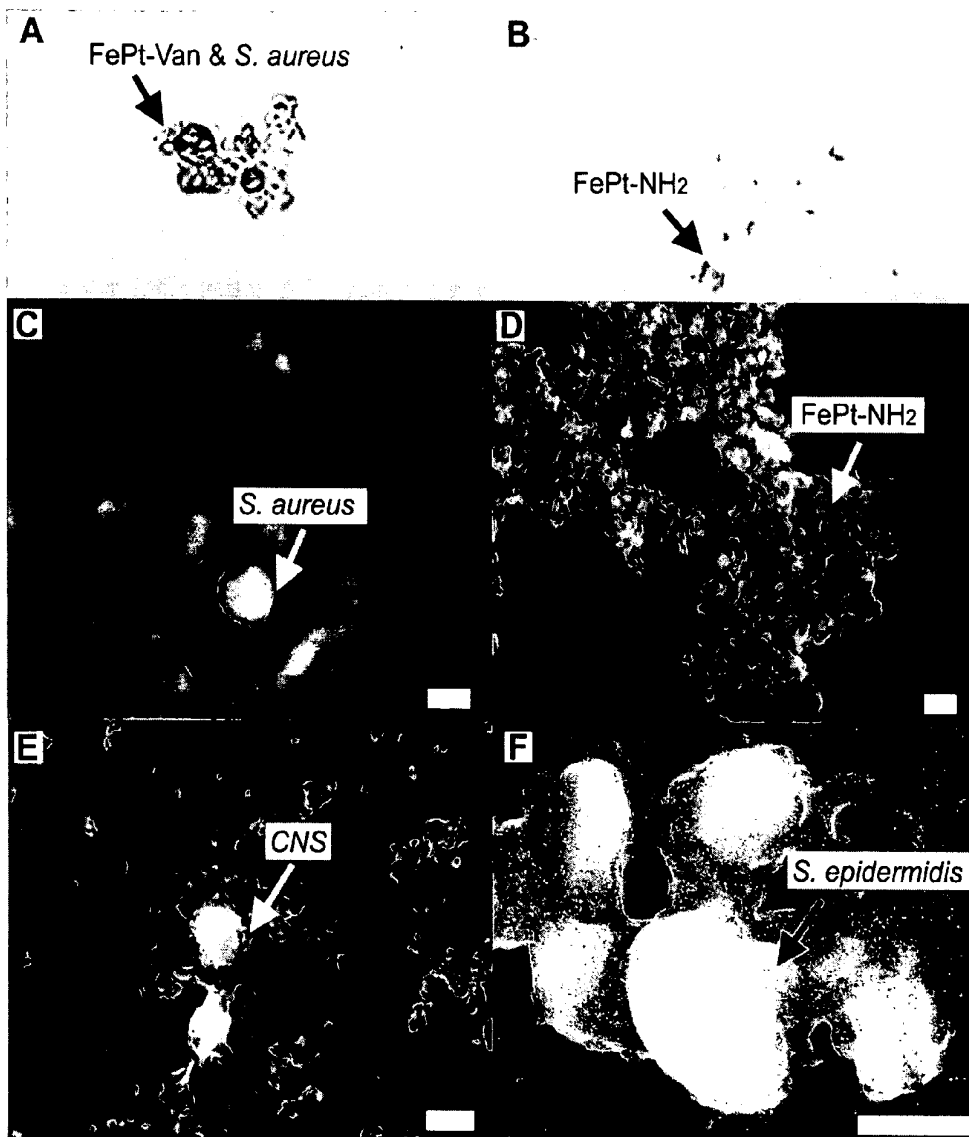


Figure 4

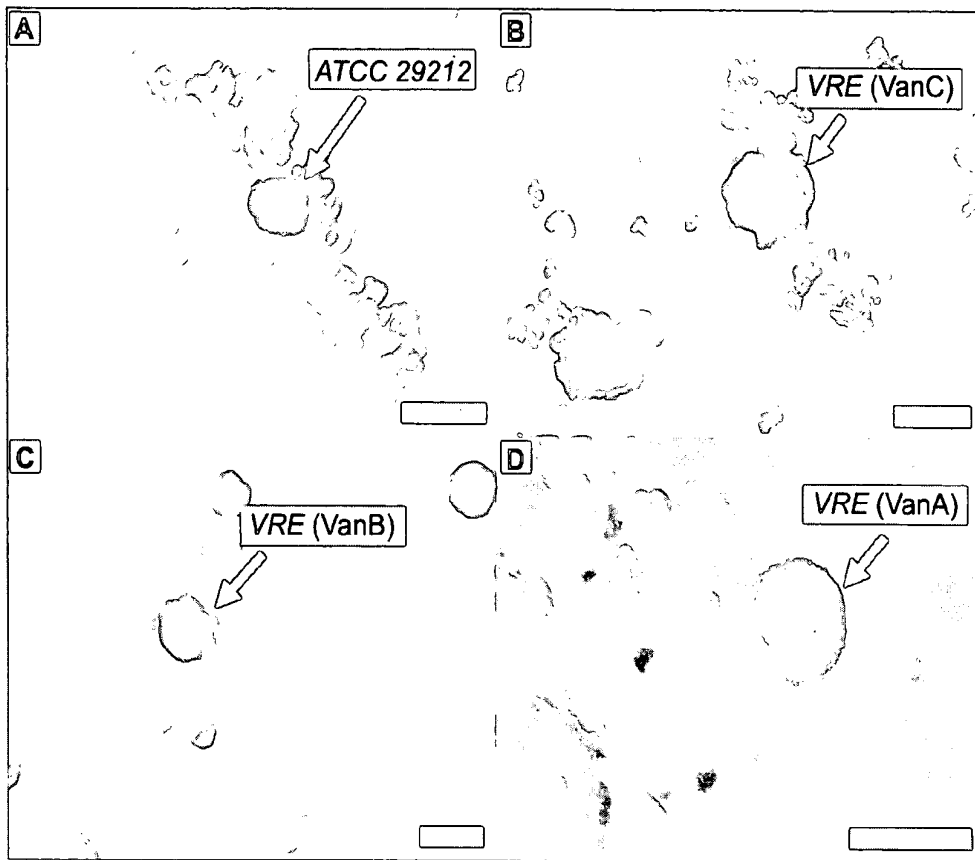


Figure 5

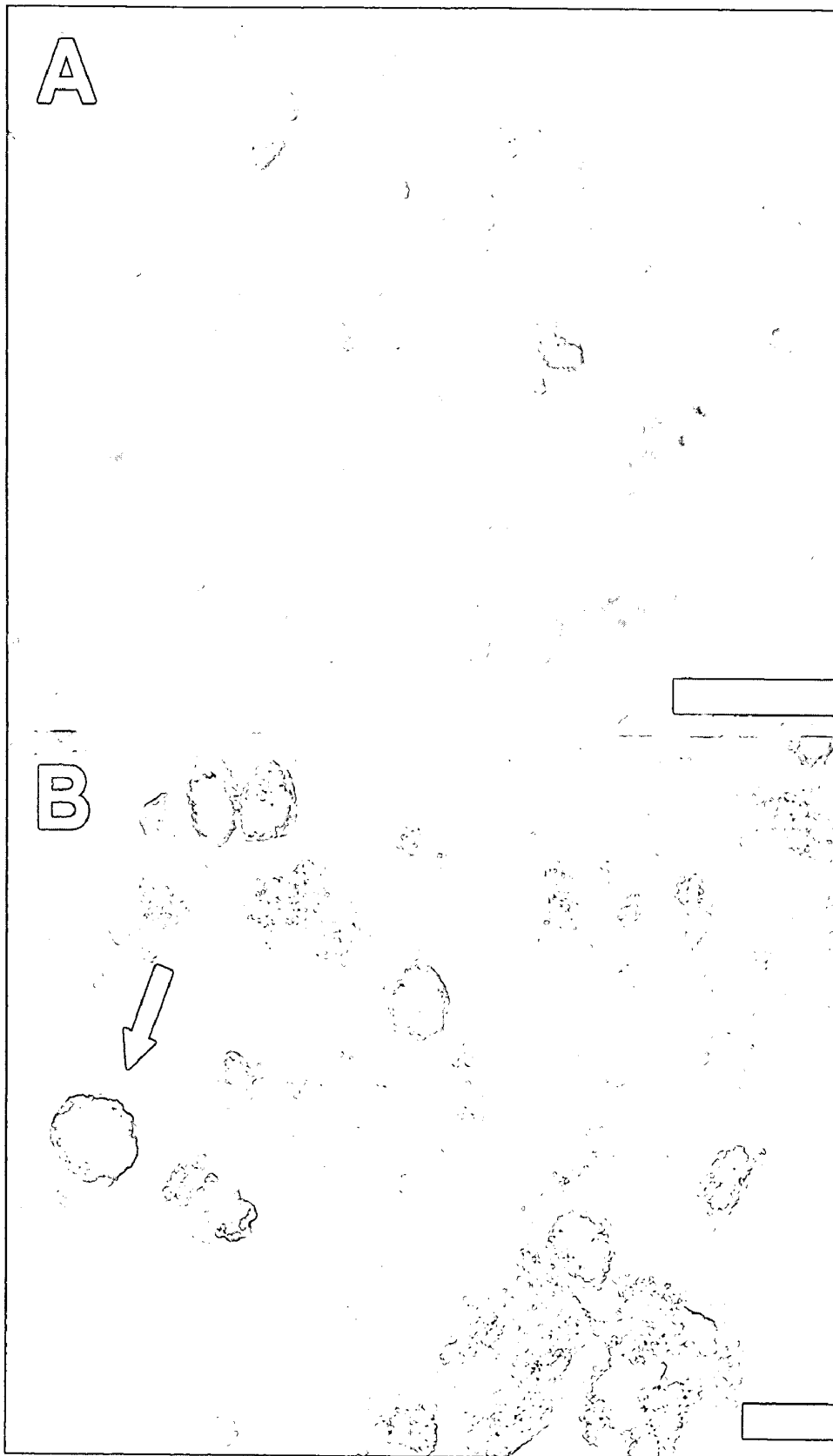


Figure 6

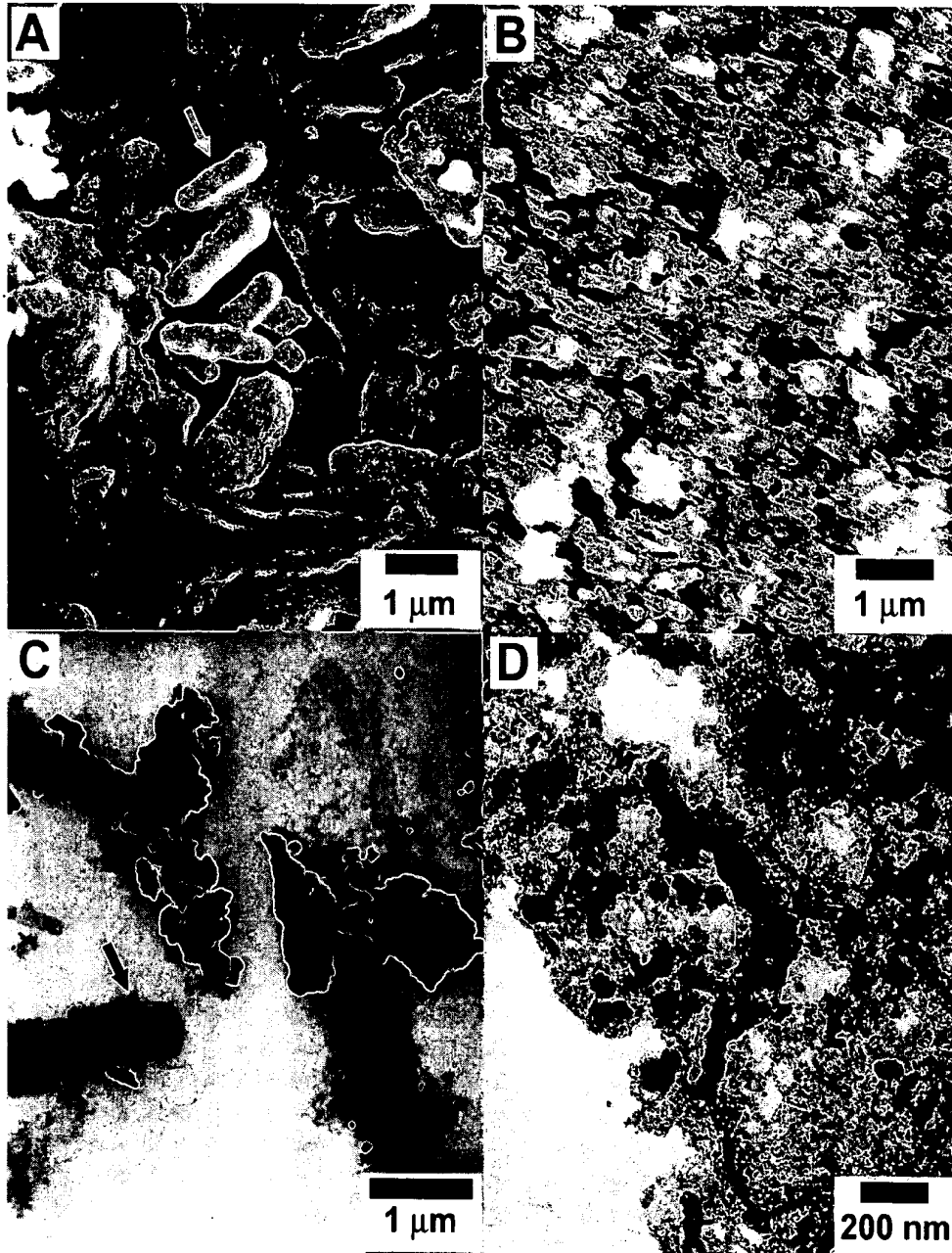
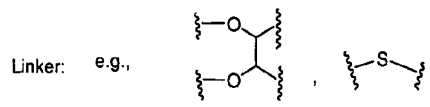
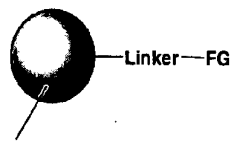


Figure 7



Magnetic nanoparticles (< 20 nm)
e.g., FePt, SmCo₅, Fe₃O₄, Fe₂O₃, etc

Function group (FG): Vancomycin, antibiotics, ligands, receptors, metal complex, etc.

one or more copies of -linker-FG covalently bond to magnetic nanoparticles

Figure 8

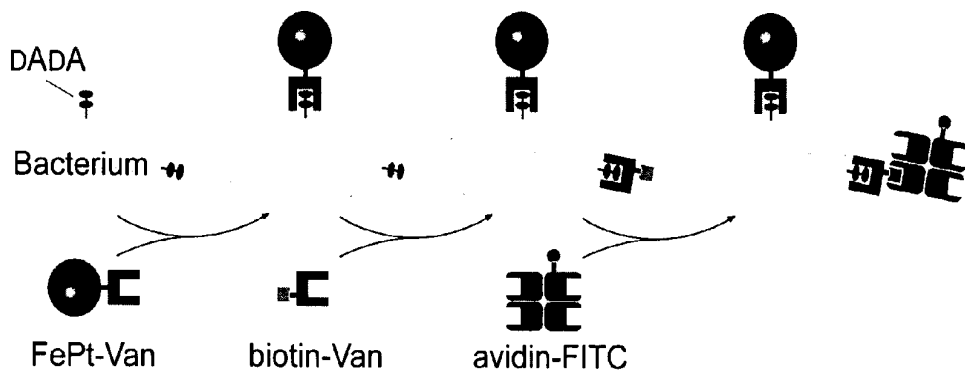
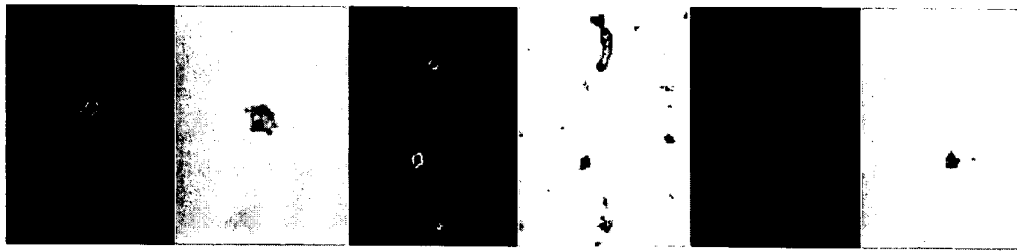


Figure 9



CNS
6 cfu/ml

Strept. pneumoniae
4 cfu/ml

control

BIOFUNCTIONAL MAGNETIC NANOPARTICLES FOR PATHOGEN DETECTION

[0001] This application claims the benefit of U.S. Ser. No. 60/582,700, filed Jun. 24, 2004, the contents of which are incorporated herein in its entirety by reference.

[0002] Throughout this application, various references are cited and disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

BACKGROUND OF THE INVENTION

[0003] Instant and sensitive detection of pathogens (e.g., bacteria, viruses) at ultra-low concentrations without time consuming procedures, such as incubation or amplification by polymerase chain reaction (PCR) offers obvious clinical benefits. However, there are, to date, no general and satisfactory assays that can detect bacteria at concentrations of $<10^7$ colony forming units per milliliter (cfu/mL) without pre-enriching the bacteria via a culture process.¹

[0004] 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

SUMMARY OF THE INVENTION

[0005] The present invention discloses a method of detection of microorganisms, such as pathogens, at ultra-low concentrations without time consuming procedures, such as culture process or polymerase chain reaction (PCR). The present invention discloses a method that uses biofunctional magnetic nanoparticles to capture and detect vancomycin-resistant enterococci (VRE) and other Gram-positive or Gram-negative bacteria at concentrations of at least as low as 10 cfu/mL within an hour, which is an order of magnitude more sensitive than the current best assay for bacteria detection based on luminescence (detection limit: 180 cfu/mL). This invention offers benefits in clinical diagnosis, food quality monitoring, environmental protection, and bioterrorism prevention.

[0006] The present invention takes advantage of the large surface/volume ratio of magnetic nanoparticles and the concept of ligand-receptor interactions for fast pathogen detection. The method combines two kinds of interactions: 1) magnetic dipole interactions that aggregate the magnetic nanoparticles under a magnetic field and 2) specific multiple ligand-receptor interactions (i.e., polyvalent interactions⁶) that offer high avidity between magnetic nanoparticles and bacteria when the ligands covalently bond to the magnetic nanoparticles.

[0007] For example, vancomycin (Van), an antibiotic, can be attached to the surface of FePt (iron-platinum) nanoparticles³ (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 (FIG. 1),^{7,8} and previous studies have demonstrated that multivalent Vans have high affinities toward multiple D-Ala-D-Ala receptors.⁹ The designed conjugate of Van and FePt magnetic nanoparticle

(FePt—Van) exhibited high sensitivity to bacteria whose cell walls express D-Ala-D-Ala as the terminal peptides, and captured those Gram-positive bacteria at a concentration as low as ~ 4 cfu/mL. More importantly, this invention shows that the designed conjugate of Van and FePt magnetic nanoparticle (FePt—Van) effectively captures VRE, a life-threatening pathogen, at a concentration of 10^1 - 10^2 cfu/mL via polyvalent interactions, which cannot be done by microbeads.

[0008] After capture of the bacteria by the magnetic nanoparticles, the resulting bacteria-nanoparticle complex can be aggregated using a magnetic field. Detection can involve analysis with an optical microscope, an electron microscope, or a combination thereof. The reduction of particle size from micrometers to nanometers allows for increased sensitivity (i.e. capture of bacteria at concentrations as low as 4 cfu/ml) and specificity (i.e. capture of bacteria only).

[0009] In another embodiment, after the bacteria has been captured by the magnetic nanoparticles, a conjugate of Van and biotin (Van-Biotin) can be used to further bind to the free D-Ala-D-Ala on the surface of the bacteria. This is beneficial, because the magnetic nanoparticles will not exhaust all the D-Ala-D-Ala (or other biomarkers) on the surface of the bacteria. Thus, the free D-Ala-D-Ala (or other biomarkers) are available for binding with a second ligand, a biotin conjugate such as Van-biotin. By exploiting the interaction between biotin and avidin, the presence of the bacteria can then be easily reported using conventional fluorescence or enzyme-linked immunosorbent assays (ELISA). Although Van-biotin was the conjugate used in this embodiment, any conjugate that contains a ligand that will bind to a pathogen and a tag (e.g., biotin, which can bind to fluorescent avidin) that will bind to a fluorescent marker can be used.

DETAILED DESCRIPTION OF THE FIGURES

[0010] FIG. 1. An illustration shows the synthesis of Van-FePt nanoparticle 2 and control FePt nanoparticle 4 conjugates.

[0011] FIG. 2. An illustration shows the capture of bacteria by vancomycin-conjugated magnetic nanoparticles in 2A via multivalent interaction and the corresponding control experiment in 2B.

[0012] FIG. 3. An optical image 3A and a SEM image 3C show the aggregates of *S. aureus* and Van-FePt nanoparticles; an optical image 3B and a SEM image 3D show the aggregates of control FePt nanoparticles after mixing with *S. aureus*; SEM image 3E shows the aggregates of coagulase negative staphylococci (CNS) and Van-FePt nanoparticles; and SEM image 3F shows *S. epidermidis* and Van-FePt nanoparticles (magnification for a and b=400, scale bars=1 μm ; the exact counts of the bacteria were confirmed by back titration).

[0013] FIG. 4. SEM images are shown of *E. faecalis* (ATCC 29212, a Van-sensitive strain, 26 cfu/mL) in 4A, *E. GALL* (a VanC strain, 84 cfu/mL) in 4B, *E. faecium* (a VanB strain, 22 cfu/mL) in 4C, and *E. faecium* (a VanA strain, 34 cfu/mL) in 4D and the aggregates of Van-FePt nanoparticles (scale bars=1 μm , the genotypes of the strains were determined by PCR, and the exact counts of the bacteria were confirmed by back titration).

[0014] FIG. 5. SEM images are shown of the aggregate of Van-FePt nanoparticles (scale bar=1 μm) in 5A and the aggregate of Van-FePt nanoparticles and *S. epidermidis* (indicate by the arrow, scale bar=2 μm) in 5B.

[0015] FIG. 6. SEM images are shown of the aggregates of *E. coli* and Van-FePt nanoparticles (arrow indicates the *E. coli*) in 6A and the aggregates of control FePt nanoparticles in 6B. TEM images are shown of aggregates of *E. coli* and Van-FePt nanoparticles (arrow indicates the *E. coli*) in 6C and aggregates of control FePt nanoparticles in 6D.

[0016] FIG. 7. An illustration shows the general structure of the biofunctional magnetic nanoparticles with one or more copies of the “-Linker-FG” (function group) covalently bonded to the magnetic nanoparticles. Magnetic nanoparticles (<20 nm) include, but are not limited to, FePt, SmCo_5 , Fe_3O_4 , Fe_2O_3 , FePd, CoPt, $\text{Sm}_x\text{Co}_y@Fe_2O_3$, $\text{Sm}_x\text{Co}_y@Fe_3O_4$, $M@Fe_2O_3$, or $M@Fe_3O_4$, whereby $x=1$ to 4, $y=5$ to 20, and M is a magnetic metal selected from the group consisting of cobalt, nickel, iron, and magnetic alloys thereof. Function groups (FG) include, but is not limited to, Vancomycin (Van), antibiotics, ligands, receptors, or metal complexes.

[0017] FIG. 8. A schematic illustration shows the mechanism for an on-particle fluorescence or ELISA assay.

[0018] FIG. 9. The images show the results of on-particle fluorescence reporting of the presence of captured bacteria.

DETAILED DESCRIPTION OF THE INVENTION

[0019] This invention provides a method of detecting microorganisms comprising steps of: (a) contacting a sufficient amount of biofunctional magnetic nanoparticles with an appropriate sample for an appropriate period of time to permit the formation of complexes between the microorganisms and nanoparticles; (b) using a magnetic field to aggregate said complexes; and (c) detecting said complexes.

[0020] The sensitivity of detection for the method is at least as low as 10 colony forming units (cfu) of the microorganisms in one milliliter of solution. On the upward end, the method of the present invention is capable of detecting anywhere from about 20, 40, 60, 80, or 100 cfu/mL. For viruses, the method of the present invention is capable of detecting concentrations at least as low as 10 plaque forming units per one milliliter of solution (pfu/mL) and upwards of about 100, 500, or 1000 pfu/mL.

[0021] In one embodiment, the microorganisms are pathogens. As used herein, pathogens are defined as any disease-producing microorganism. Pathogens include, but are not limited to, bacteria, viruses, mycoplasma, algae, amoeba, or other single-cell organisms. The bacteria may be either Gram positive or Gram negative, which may be captured at the same time by the present invention. The bacteria used in the present invention include, but are not limited to, *Staphylococcus aureus*, *Staphylococcus epidermidis*, coagulase negative staphylococci (CNS), *E. coli*, or Vancomycin Resistant Enterococci (VRE).

[0022] The sample to be tested can be a clinical sample, which may include, but is not limited to, bodily fluid samples, smear samples, or swab samples. The sample can also be taken from the environment, which may include, but

is not limited to, water, air, or soil. The methods of pathogen detection and reduction, as discussed herein, may be applied to the residual components of bioterrorist chemical devices.

[0023] Additionally, the sample can be taken from food products, which may include, but is not limited to, liquid or solid foods that processed, concentrated, or otherwise artificially modified. The present invention can be very beneficial to the food industry where sensitive detection of pathogens is desired. Samples, whether in solid, liquid, or gas form, can be prepared accordingly (e.g. dilution, dissolution, immersion) so as to render them in solution form for use in the present invention.

[0024] As used herein, biofunctional means the ability to engage in ligand-receptor, antibody-antigen, ionic, or metal-ligand interaction. Thus, biofunctional describes the types of specific interactions possible between the magnetic nanoparticles and microorganisms in the sample. The biofunctional magnetic nanoparticles are a conjugate of magnetic nanoparticles and a functional group.

[0025] The magnetic nanoparticles may be composed of, but are not limited to, iron, noble metals (such as gold, silver, platinum, or palladium), cobalt, metal oxides, nickel, or alloys thereof. In one embodiment, the magnetic nanoparticles are iron-platinum (FePt), SmCo_5 , Fe_3O_4 , Fe_2O_3 , FePd, CoPt, $\text{Sm}_x\text{Co}_y@Fe_2O_3$, $\text{Sm}_x\text{Co}_y@Fe_3O_4$, $M@Fe_2O_3$, or $M@Fe_3O_4$, whereby $x=1$ to 4, $y=5$ to 20, and M is a magnetic metal selected from the group consisting of cobalt, nickel, iron, and magnetic alloys thereof. In the embodiment described above involving $\text{Sm}_x\text{Co}_y@Fe_2O_3$, $\text{Sm}_x\text{Co}_y@Fe_3O_4$, $M@Fe_2O_3$, and $M@Fe_3O_4$, the symbol “@” merely indicates that the magnetic nanoparticles have a Sm_xCo_y or M core and a Fe_2O_3 or Fe_3O_4 shell. Metals which can be magnetic are well known in the art. See *Magnetic Nanoparticles Having Passivated Metallic Cores*, U.S. Ser. No. 10/355,162, filed on Jan. 31, 2003, published on Oct. 9, 2003. The functional group may be an antibiotic, ligand, receptor, or metal complex. In one embodiment, the antibiotic is vancomycin (Van).

[0026] After capture of the bacteria by the magnetic nanoparticles, the resulting bacteria-nanoparticle complex can be aggregated using a magnetic field. Detection can involve analysis with an optical microscope, an electron microscope, or a combination thereof. The reduction of particle size from micrometers to nanometers allows for increased sensitivity (i.e. capture of bacteria at concentrations at least as low as 10 cfu/mL and at least as low as 4 cfu/mL for Gram positive bacteria) and specificity (i.e. capture of bacteria only).

[0027] In another embodiment, after the bacteria has been captured by the magnetic nanoparticles, a conjugate of Van and biotin (Van-Biotin) can be used to further bind to the free D-Ala-D-Ala on the surface of the bacteria. This is beneficial, because the magnetic nanoparticles will not exhaust all the D-Ala-D-Ala (or other biomarkers) on the surface of the bacteria. Thus, the free D-Ala-D-Ala (or other biomarkers) are available for binding with a second ligand, a biotin conjugate such as Van-biotin. A magnetic field can then be used to aggregate the resulting complexes. By exploiting the interaction between biotin and avidin via an avidin-FITC (fluorescein isothiocyanate) conjugate, the presence of the bacteria can then be easily reported using conventional fluorescence or enzyme-linked immunosor-

bent assays (ELISA). Detection may further comprise analysis with an optical microscope, an electron microscope, or a combination thereof.

[0028] The present invention also provides a method of reducing the amount of pathogens in a sample, comprising the steps of (a) contacting a sufficient amount of biofunctional magnetic nanoparticles with an appropriate sample for an appropriate period of time to permit the formation of complexes between the pathogens and magnetic nanoparticles; and (b) using a magnetic field to aggregate said complexes; and (c) removing said complexes, thereby reducing the amount of said pathogen in the sample.

[0029] Additionally, the present invention provides a method for the reduction of pathogens in the bodily fluid of a subject in need of treatment thereof, comprising the steps of (a) obtaining bodily fluid from said subject; (b) contacting said fluid with biofunctional magnetic nanoparticles capable of binding to said pathogens under conditions permitting the formation of complexes between the pathogens and the magnetic nanoparticles; and (c) removing said complexes, thereby reducing the amount of pathogens in said fluid. The method may further comprise the reintroduction of said fluid back to said subject. In one embodiment, said fluid is blood.

[0030] The method of the present invention can be used to treat infections in a subject in need thereof. Particularly, this process may be adapted for ex vivo treatment. Bodily fluids from the subject can be extracted to a device containing the biofunctional magnetic nanoparticles under conditions that permit the formation of pathogen-nanoparticle complexes as described supra. After said process, the fluids may be introduced back to the subject.

[0031] In one embodiment, the subject is a mammal. Such mammal may be, but is not limited to, a dog, cat, rabbit, mouse, or human being. In another embodiment, the subject is a non-mammal, such as a bird, turtle, snake, or fish.

[0032] This invention provides for a composition capable of detecting or reducing microorganisms or in combination thereof, comprising an appropriate amount of biofunctional magnetic nanoparticles capable of binding to said microorganisms via ligand-receptor, antibody-antigen, ionic, or metal-ligand interaction. In one embodiment, the microorganisms are pathogens.

[0033] This invention also provides for a kit for detecting or reducing pathogens, comprising a compartment containing an appropriate amount of biofunctional magnetic nanoparticles capable of binding to said pathogens.

[0034] Additionally, this invention provides a device which performs the method for detecting or reducing microorganisms, comprising any of the above methods. Furthermore, a computer may be used to monitor the above-discussed methods of the present invention.

[0035] The invention can be better appreciated by reference to the Experimental Details which follow. Those ordinarily skilled in the art will readily appreciate that the specific experiments detailed are only illustrative and are not meant to limit the invention as described herein.

Experimental Details: Materials and Methods

[0036] Bacteria strains: Except ATCC 29212, the bacteria strains were obtained from clinical specimens. The *S. aureus*

and CNS were isolated from blood culture of patients admitted to the Queen Mary Hospital, Hong Kong. The VRE were isolated from rectal swabs of patients admitted to Queen Mary Hospital, Hong Kong.

[0037] Establishment of the counts of the bacteria: Bacterial cells were suspended in saline and adjusted to the 0.5 McFarland standard (approximately 1 to 2×10^8 cfu/mL). Serial ten-fold dilutions were made until the desired inoculum density was reached. The actual count was determined by back titration. A sample of each inoculum was streak onto a blood agar plate. The dilution that yields 30 to 100 cfu/mL in the agar plate was counted for calculating the actual inoculum density. All tests were performed in triplicates.

[0038] Synthesis of biofunctional magnetic nanoparticles: **FIG. 1** illustrates the synthetic route for making the FePt—Van nanoparticles: Bis(Vancomycin) cystamide (in aqueous solution) reacts with FePt nanoparticles (in hexane phase) under vigorous stir for 12 hours to form Pt—S and Fe—S bonds that link Van to FePt. To ensure all the Vans to be completely consumed, FePt is slightly in excess. After the reaction completes, the FePt—Van nanoparticles **2** dissolve into the aqueous phase, which can be easily separated from the organic phase. The UV-vis spectrum of the aqueous solution of FePt—Van nanoparticles **2** exhibits an absorption at ~ 280 nm (originated from the phenyl groups of Van), indicating that Van has attached to the FePt nanoparticles. X-ray Photoelectron Spectroscopy (XPS) of the FePt—Van nanoparticles **2** shows peaks at 72.6 eV and 713/726 eV, corresponding to the energies required for breaking Pt—S and Fe—S bonds, respectively. Time-of-Flight Secondary Ion Mass spectra (ToF—SIMS) of the FePt—Van nanoparticles **2** displays mass peaks at 88 (FeS^+) and 1506 (Van^+), confirming the presence of Van on the surface of FePt. Each FePt nanoparticle has 8–9 Vans on its surface calculated according to the calibration curves generated using the solutions of Van and FePt with known concentrations. As a control, FePt nanoparticles react with cystamine (**FIG. 1**) to give FePt— NH_2 **4**. The XPS of FePt— NH_2 **4** also displays peaks of Pt—S (72.5 eV) and Fe—S (713/726 eV) bonds, and the ToF—SIMS of FePt— NH_2 **4** shows mass peaks at 76 ($\text{NH}_2\text{CH}_2\text{CH}_2\text{S}^+$) and 88 (FeS^+), further confirming the 2-amino-ethanethiol on the surface of FePt.

[0039] Typical experimental procedure for capture (**FIG. 2**): The bacteria solution was diluted to the concentration of ~ 10 cfu/mL in PBS buffer. FePt—Van solution was filtered through a $0.45 \mu\text{m}$ filter to eliminate the possible bacteria contamination and aggregated FePt—Van. The concentration of the filtrated FePt—Van solution was $11 \mu\text{g/mL}$. $250 \mu\text{L}$ of the FePt—Van solution was added to $500 \mu\text{L}$ of the above bacteria buffer solution, and was mixed in a 1 mL vial for 0.5 minute. After standing still for 10 minutes to allow FePt—Van to attach to the surface of the bacteria, a ~ 400 G magnet was put outside of the vial to pull the “magnetized” bacteria to the side-wall of the vial. After applying magnetic field for another 10 minutes, solution was removed. The aggregates attracted by the magnet were washed by deionized (DI) water before being transferred for analysis by optical microscope and electron microscope.

Capture of Gram Positive Bacteria:

[0040] Experimental results showed that FePt—Van nanoparticles **2** indeed captured Gram-positive strains such as *S. aureus* (8 cfu/mL), *S. epidermidis* (10 cfu/mL), and a

coagulase negative staphylococci (CNS, 4 cfu/mL). **FIG. 3A** shows the optical image of the isolated *S. aureus* when FePt—Van nanoparticles **2** are used, and the scanning electron micrograph (SEM) confirms that these “magnetized” *S. aureus* aggregate with FePt—Van nanoparticles **2** under magnetic field (**FIG. 3C**). When FePt—NH₂**4** is used, neither the optical microscopy (**FIG. 3B**) nor the SEM (**FIG. 3D**) shows *S. aureus*, suggesting that FePt—Van nanoparticles **2** bind to *S. aureus* specifically due to the molecular recognition. FePt—Van nanoparticles **2** and FePt—NH₂**4** were used to treat solutions containing other Gram-positive strains, such as *S. epidermidis* or CNS. Experimental results showed that FePt—Van nanoparticles **2** capture both *S. epidermidis* and CNS, but FePt—NH₂**4** captures neither of them, which further confirms the binding of FePt—Van nanoparticles **2** to the Gram-positive bacteria via specific interaction, similar to the case of *S. aureus*. **FIGS. 3E and 3F** show the SEM images of those bacteria and the aggregates of FePt—Van nanoparticles **2**.

Capture of VRE Bacteria

[0041] The process here is similar to the one above. Despite the fact that the affinity of Van to the terminal peptides on the cell wall of VRE decreases due to mutation,⁷ previous study shows that Van-decorated gold nanoparticles¹¹ bind strongly to VRE. Similarly in the current experiment, FePt—Van nanoparticles **2** bind to VRE with high avidity via polyvalent interactions and captured VRE. **FIG. 4** 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 FePt—Van nanoparticles **2** binds to the surface of the VRE cell. These results not only further demonstrate the effectiveness of FePt—Van nanoparticles **2** as a polyvalent ligand, but also provide a useful method to capture VRE.

[0042] Capture bacteria in the present of WBC: After adding FePt—Van nanoparticles **2** into the solution of white blood cells (WBC, 100 cfu/mL) and following the capture experimental procedure, SEM shows only the aggregates of FePt—Van nanoparticles **2** (**FIG. 5A**), indicating no binding between WBC and FePt—Van nanoparticles **2**. When FePt—Van nanoparticles **2** was added to the mixture of WBC (100 cfu/mL) and *S. epidermidis* (15 cfu/mL) and applied the capture procedure, SEM shows only the cells of *S. epidermidis* (**FIG. 5B**) and the aggregates.

Capture of Gram Negative Bacteria (**FIG. 6**)

[0043] After adding the aqueous solution of FePt—Van nanoparticles **2** (13 µg/mL) or FePt—NH₂**4** (15 µg/mL) into a vial of solution containing *E. coli* (15 cfu/mL) and shaking for 20 minutes, magnetic field (~3000 G) was applied to the solution. Upon the application of magnetic field, the magnetic nanoparticles aggregated irreversibly due to the high magnetic anisotropy of the FePt nanoparticles. Although this was sometimes an unwanted phenomena in the synthesis of magnetic nanoparticles (e.g., FePt or SmCo₅),^{3,10} it turned out to be very useful for increasing the load of magnetic nanoparticles attached to the bacteria (vide infra), thus providing adequate force to “focus” the bacteria into a small area when a small magnet is used. Then the remaining solution was removed and the aggregates were washed thoroughly using deionized water and transferred to a glass slide for microscopic study.

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What is claimed is:

1. A method of detecting pathogens in a sample, comprising the steps of:

- (a) contacting biofunctional magnetic nanoparticles with a sample, to form pathogen-nanoparticle complexes;

- (b) using a magnetic field to aggregate said complexes; and
- (c) detecting said complexes.
2. The method of claim 1, wherein the biofunctional magnetic nanoparticles are capable of engaging in ligand-receptor, antibody-antigen, ionic, or metal-ligand interaction.
3. The method of claim 1, wherein the biofunctional magnetic nanoparticles are a conjugate of magnetic nanoparticles and a functional group.
4. The method of claim 3, wherein the magnetic nanoparticles are iron, noble metals, cobalt, metal oxides, nickel, or alloys thereof, and the functional group is an antibiotic, ligand, receptor, or metal complex.
5. The method of claim 4, wherein the noble metals are selected from the group consisting of gold, silver, platinum, and palladium.
6. The method of claim 4, wherein the magnetic nanoparticles are FePt, SmCo₅, Fe₂O₃, Fe₃O₄, FePd, CoPt, Sm_xCo_y@Fe₂O₃, Sm_xCo_yFe₃O₄, M@Fe₂O₃, or M@Fe₃O₄, whereby x=1 to 4, y=5 to 20, and M is a magnetic metal selected from the group consisting of cobalt, nickel, iron, and magnetic alloys thereof.
7. The method of claim 4, wherein the antibiotic is vancomycin (Van).
8. The method of claim 1, wherein the sample is a solid, liquid, or gas.
9. The method of claim 1, wherein the pathogens are bacteria or viruses.
10. The method of claim 9, wherein the bacteria are Gram positive or Gram negative.
11. The method of claim 10, wherein the bacteria are *Staphylococcus aureus*, *Staphylococcus epidermidis*, coagulase negative staphylococci, *E. coli*, or Vancomycin Resistant Enterococci.
12. The method of claim 1, wherein detection involves analysis with an optical microscope, an electron microscope, or a combination thereof.
13. The method of claim 1, wherein said method detects pathogens at a concentration of at least as low as 10 colony forming units (cfu) in one milliliter of solution.
14. The method of claim 1, wherein said method detects pathogens at a concentration of at least as low as 4 colony forming units (cfu) in one milliliter of solution.
15. The method of claim 1, adopted for the reduction of pathogen in the bodily fluid of a subject in need of treatment thereof, further comprising the step of removal of said complexes.
16. The method of claim 15, further comprising the reintroduction of said fluid back to the body of said subject.
17. The method of claim 15, wherein the bodily fluid is blood.
18. The method of claim 15, further comprising the monitoring of said method by a computer.
19. A method of detecting pathogens in a sample, comprising the steps of:
- (a) contacting biofunctional magnetic nanoparticles with a sample to form pathogen-nanoparticle complexes;
 - (b) contacting the pathogen-nanoparticle complexes with biotin conjugates;
 - (c) using a magnetic field to aggregate said complexes; and
 - (d) detecting said complexes.
20. The method of claim 19, wherein detection comprises a fluorescence assay, an enzyme-linked immunosorbent assay (ELISA), analysis with an optical microscope, analysis with an electron microscope, or a combination thereof.
21. A composition for detecting pathogens, comprising an appropriate amount of biofunctional magnetic nanoparticles capable of ligand-receptor, antibody-antigen, ionic, or metal-ligand interaction with said pathogens.
22. A kit for detecting pathogens, comprising a compartment containing the composition of claim 21.

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