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Innovative Developments in Bacterial Detection with Magnetic Nanoparticles

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Abstract It has been seen from the last decade that many bacterial strains are becoming insensitive to conventional detection techniques and it has its own limitations. Current developments in nanoscience and nanotechnology have expanded the ability to design and construct nanomaterials with targeting, therapeutic, and diagnostic functions. These multifunctional nanomaterials have attracted researchers, to be used as the promising tool for selective bacterial sensing applications. An important advantage of using magnetic nanoparticles to capture bacteria is the simple separation of bacteria from biological samples using magnets. This review includes significance of magnetic nanoparticles in bacterial detection. Relevant to topic, properties, designing strategies for magnetic nanoparticle, and innovative techniques used for detection are discussed. This review provides the readers how magnetic properties of nanoparticles can be utilized systematically for bacterial identification.

Keywords Magnetic nanoparticles (MNPs) · Bacteria detection · Colony forming units · Surface functionalization

Introduction

Bacteria are one of the ubiquitous living creatures on earth, having adapted to all available ecological habitats. Bacteria show biodiverse impact on ecological system as symbionts and parasites. They benefit their host and have economic importance in food, agricultural, pharmaceutical, petroleum industries, etc. But they also show pathogenicity to human and other living effects. Unwanted presence of bacteria in natural resources makes it difficult to use. Great concern is given for studies of interactions of bacteria with ecosystem. For this, the most prerequisite is

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detection of bacteria. Bacterial detection is used in a wide variety of applications including biohazard, clinical diagnosis, microbial forensic, environmental studies, and many more [1].

Detection of bacteria is based upon fundamental characteristics like morphological and/or molecular chemistry of cells. Many conventional and advanced systems have been established for the detection of bacteria. Among these systems, conventional methods are based upon morphological and biochemical analysis, which before detection require intermediate processes such as extraction, isolation, culturing, counting, etc., resulting in time consuming, tedious, and difficult on-site diagnosis. Additionally, microorganisms are consistently resisting towards conventional chemicals, drugs, and analytical media. All this prompts search for newer alternatives [1, 2]. Pathogenic bacteria are major concerns regarding human health, food industries, and water facilities. Accurate and definitive microorganism identification including bacterial identification and detection is essential for correct disease diagnosis, treatment of infection, and trace-back of disease outbreaks associated with microbial infections. For this, innovative, rapid, sophisticated, high sensitive detection methods are demanded. Successful attempts are made to develop molecular analyzing techniques like ELISA, PCR, ribotyping, micro array, etc. Aside from high sensitivity and reliability, these techniques suffer from high cost of performance, sample pretreatment, and lower limit of detection [3, 4]. The drawbacks of conventional and current molecular diagnostics can be overcome by assisting them with nanoscience.

Nanotechnology is a multidisciplinary branch of science, which deals with technology relating to nanosize materials. It has a huge significance in biomedical, pharmaceutical, agricultural, environmental, and many more branches of science. Nanobiotechnology is a branch where nanomaterials are fabricated and used for biological and biochemical applications. It demonstrates all facets of research of biology assisted with nanotechnology [5]. In the properties of sub 100-nm materials and device, their surface modification has contributed massively to biomedical fields such as cellular repair, drug delivery, therapeutic applications, and diagnostic aids [6]. Knowledge and application of nanomaterial make in depth all bimolecular processes easy to understand. Although many techniques are still in the nascent stage of development, some are actually being employed in daily practices [7]. Use of nanobiotechnology extend the limits of current molecular diagnostics, allows point-of-care diagnostics, and integrates diagnostics with therapeutics. It enables diagnosis at single-cell and molecule level [8]. Magnetic nanoparticles (MNPs) possessing nanoscale size range are examples of bionanomaterial, which mimics the size of molecules in nature, and possess favorable characteristics, which made them multifunctional regarding bionanoapplications. The use of high surface area and superparamagnetic property of MNPs provides a promising and sophisticated platform for detecting techniques so that conventional and molecular diagnostics become much easier and more worthy [9] (Fig. 1).

Bacteria and Its Detection

With respect to conventional sense of biology, bacteria are known as microscopic organisms, ranging in few micrometers in length, of wide spread variety. They are unicellular, prokaryotic in nature with no internal organization, and multiply by undergoing fission. They differ in shapes like rod, spherical, and cuboidal. They arranged themselves in single, pair, chain, and cluster form. They have a single chromosome with a close circle of double-stranded DNA. Sometimes, they possess characteristic appendages like flagella. Cell wall is rigid and made up of phospholipid bilayers. Bacteria can be

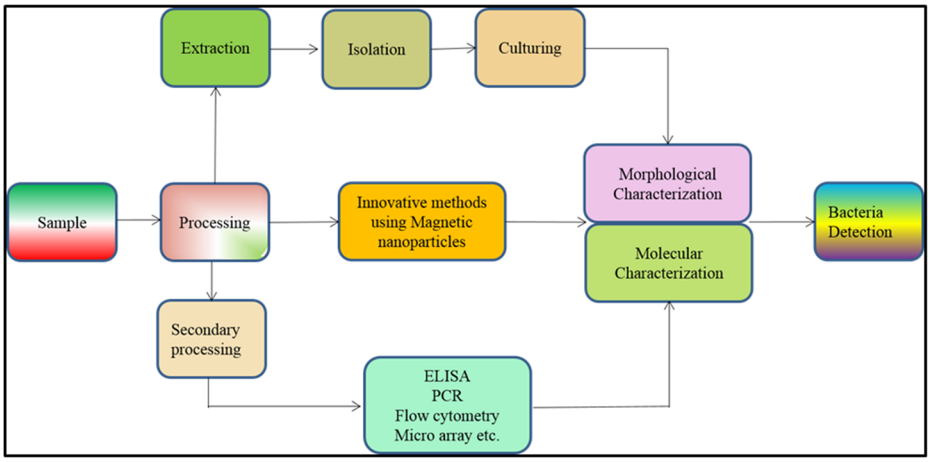

Fig. 1 Rapid and real-time detection of bacteria by using MNPs

categorized on different bases like type of staining, e.g., gram-positive, gram-negative, culturing requirements aerobic, anaerobic, etc. Mostly, they are recognized on the basis of gram staining. Gram-positive bacteria possess a thick cell wall containing many layers of peptidoglycan and teichoic acid. In contrast, gram-negative bacteria have a relatively thin cell wall consisting a few layers of peptidoglycan surrounded by a second lipid layer containing lipopolysaccharides and lipoproteins [1]. Zeta potential at the interfacial region of bacterial surface helps to retrieve a net charge on cell surface. At physiological pH values, there is net negative electrostatic surface charge. In gram-positive bacteria, the peptidoglycan cell wall influences surface electro negativity by virtue of phosphoryl group in substituent teichoic and teichuronic acid residues and unsubstituted carboxylate groups. In gram-negative bacteria, phosphoryl and 2-keto-3-deoxyoctonate carboxylate groups of lipopolysaccharide located in the outer membrane confer the negative electrostatic surface [10] (Fig. 2). These fundamentals of morphology, molecular chemistry, and surface physiochemical property are important considerations while detecting bacterial species.

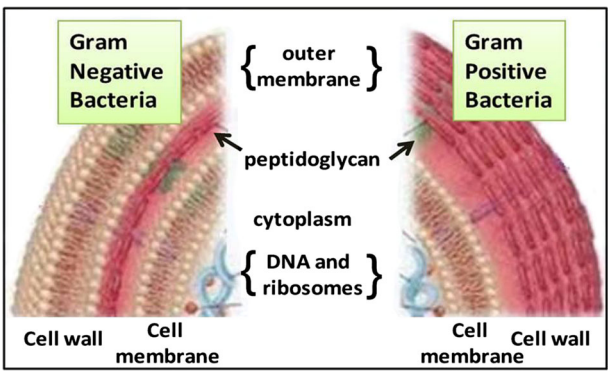

Fig. 2 General comparison of gram-positive and gram-negative bacterial cell wall structure [11]

Principles of Bacterial Detection

Detection of bacteria is based upon either morphological features or molecular chemistry of cell. All methods of detection, either conventional or recent trends, fall under a principle mentioned below.

Phenotypic method: Here, initial putative detection is done by examining microscopic characteristics, e.g., cell shape, gram staining, appearance of special structure like spore, capsule, and macroscopic characteristics, e.g., appearance of colony, speed, and pattern of growth. It also includes examination of physiological and biochemical characteristics marking presence of enzymes, capacity to metabolize various molecules, etc.

Immunological/serological method: Method interaction between bacterial antigen and its specific antibody is considered as a base for detection. It involves test for particle agglutination, direct indirect immunofluorescence, immunochromatography, optical immunoassays, etc.

Genotypic method: Organisms are detected on the basis of molecular techniques, by analyzing genetic material either DNA or RNA. Here, specific gene or a particular nucleic acid sequence is interpreted as a definitive identification of organism [3, 12].

Significance of MNPs in Bacterial Detection

The unique physiochemical properties of MNPs such as size, magnetization level, and its surface morphology are complementary for biological applications. Rapid and real-time detection with small sample volumes is possible due to large surface/volume ratio [13]. Size range of MNPs from few nanometers to tens of nanometers made them get closer to a biological entity of interest. Functionalization of MNPs enables them to bind or interact with a biological target. The large number of target-specific molecules of interest can be attached to a large surface area provided by nanoparticles for ultra-sensitive detection.

Designing of MNPs for Bacterial Detection

Synthesis Strategy

The magnetic properties of MNPs depend upon its morphology and structure, influenced by its synthesis methods and conditions. Successful attempts are made to develop good control on morphological and magnetic properties. While synthesizing MNPs, attention should be given for monodispersion and reproducibility of nanoparticles [14]. To attain this, various physical, chemical, and biological methods have been developed. Among these, chemical and biological are methods of choice to synthesize nanobiomaterial [15].

Chemical synthesis: Synthesizing nanoparticles by chemical route provides flexible designing and synthesizing of newer materials, which can be polished into the final product. Since

mixing occurs at the molecular level, it offers good chemical homogeneity. Co-precipitation, sol–gel, thermal decomposition, hydrothermal synthesis, micro-emulsion, combustion, and polyol syntheses are some of the techniques of chemical synthesis, which are used for biochemical applications [16, 17]. These methods result into similar composition and narrow size distribution of nanoparticles. Mostly for the production of MNPs, co-precipitation technique of iron salts is preferred [17].

In co-precipitation method, the salt solution of the required metallic elements is reduced by alkali solution. The co-precipitation is a two-step process, where firstly, solid hydroxides of metals in the form of colloidal particles are obtained, and secondly, hydroxides obtained from alkaline solution are heated to provide transformation of solid solution of metal hydroxides to the ferrite. Two stages are involved here: at critical super saturation of species, a short burst of nucleation occurs, and then, there is a slow growth of the nuclei by diffusion of the solutes to the surface of the crystal. To produce monodispersed magnetic NPs, these two stages should be separated, i.e., nucleation should be avoided during the period of growth. Though a large amount of NPs can be synthesized, the control on particle size distribution is difficult [18, 19].

Hydrothermal synthesis: It is performed in aqueous media by using different polyols in reactors or autoclaves under different conditions of the pressure and temperature. Hydrolysis and oxidation or neutralization of mixed metal hydroxides are the main two routes for the formation of ferrites. Solvent, temperature, and time have important effects on the products [20]. The particle size in crystallization is controlled mainly through the rate processes of nucleation and grain growth where rates depend upon the reaction temperature, with other conditions held constant [21]. Polyol method is a very promising uniform nanoparticle synthesis technique. Liquid polyols such as ethylene glycol or diethylene and triethylene glycols are used both as a solvent of metallic precursor and as a reducing agent for the chemical preparation of metallic cations from various inorganic precursors [20]. The basic reaction scheme for the synthesis of these metal powders by the polyol process involves dissolution of the solid precursor, reduction of the dissolved metallic species by the polyol itself, nucleation of the metallic phase, and growth of the nuclei [22].

Combustion synthesis (CS): It follows highly exothermic redox reaction at a low temp to produce oxides. The reaction itself acts as a power house instead of using high-temperature furnaces during post-annealing procedures for a prolonged time. In CS, exothermic reactivity is the key characteristic used to produce nanomaterials. The parameter which should be considered for combustion reactions is C/H ratio (type of fuel), the fuel to oxidizer ratio (F/O), the water content of the precursor mixture, and the ignition temperature. Enthalpy or flame temperatures generated during combustion have effects on powder characteristics such as crystallite size, surface area, size distribution, and nature of agglomeration [23, 24].

Biological synthesis: It is a clean, non-toxic, and eco-friendly method of synthesis. Nanobiotechnology demands different compositions, sizes, shapes, and controlled dispersity of NPs. Biosynthesis method for NPs can fulfill these demands, and thus, it has been emerged as a promising field of research [25, 26].

Different Surface Modifications Strategy

To obtain physically and chemically stable colloidal systems, importance should be given towards protection of MNPs since iron particles are tremendously reactive towards oxidizing agents. This problem can be overcame by surface coating of the MNPs. Coating leads to improve stability of the particles, increase water dispersibility, and provide functionalization for further conjugation with bioactive molecules or targeting ligands [17, 27]. Stabilization can be achieved by the following methods.

Surface coating: Polymeric stabilizers, e.g., dextran, carboxy-dextran, polyvinyl alcohol (PVA), or polyethylene glycol (PEG), are utilized as a coating material. Some atomic layers of inorganic metals (e.g., gold), nonmetals (e.g., graphite), or oxide surfaces can be deposited on the surface of NPs as a coating material [23, 28, 29].

Polymeric shells: It avoids cluster growth after nucleation and holds the particle apart against attractive forces. A nanosphere consists of composite particles prepared from monomers or preformed polymer matrix, and nanocapsules are an aqueous or an oily core surrounded by a polymeric shell [30].

All these methods are useful for surface functionalization of MNPs and lead to increases in their water dispersibility.

Use of Stabilizing Surface Coating Materials

Surfactants or polymers can be used during the synthesis of MNPs so that newly formed surfaces get stabilized and aggregation can be prevented. Size of the colloid is determined by type and arrangement of surface coating onto the magnetic core. Coating of NPs play an important role regarding interaction of NPs with a biological system [31]. Monomeric stabilizers of organic origin carrying functional groups like carboxylate, phosphate, or sulfate, e.g., phosphonic acids [32], oleic acid [19], lauric acid [33], or polymeric stabilizers, i.e., dextran [34], PEG (polyethylene glycol) [35], PVA [23], chitosan [36], and poly(ethylene imine) (PEI) are used as surface stabilizers. Amphiphilic polymers having a hydrophilic segment spread into the aqueous medium, and a hydrophobic segment presents onto the particle surface, resulting into the prevention of aggregation of MNPs [37]. A polymeric coating alters the surface properties of the MNPs and acts as a barrier for preventing aggregation, which brings magnetic nanofluid into a physically and chemically stable form. For such surface-modified MNPs, when used for biomedical applications, few polymer characteristics must be considered like attachment mechanism to the particle surface, biostability, chemical structure, conformation, degree of surface coverage, length, molecular weight, hydrophobicity, hydrophilicity, etc. Like PEG, the polysaccharide dextran also proves to be a good surface coating material for in vivo imaging applications [38]. Generation and functionalization of amino groups onto MNPs facilitate attachment of biomolecules onto the particle surface [22, 39]. Monolayers of alcohol or carboxylic acid terminated can be deposited to gold, which results into surface functionalization with ligands of interest [29, 40]. Coating of gold to MNPs shows good stabilization under acidic and neutral pH in aqueous media. Alkoxysilane functionalized and SiO_2-coated MNPs facilitate stability of NPs and make them applicable for different biofunctions [41, 42].

MNPs Encapsulated in Polymeric Shell

Encapsulation of MNPs in polymeric shell configures a core shell-like structure, which modifies the water dispersion and physical and chemical stability of the colloid. Nowadays, up to a greater extent, natural and synthetic biodegradable polymers like human serum albumin [43], chitosan [44], acropol [45], poly(methyl methacrylate) (PMMA) [27] are used as an encapsulating material. Functional groups such as terminal amine or carboxyl are provided by a polymeric shell on MNP surface for further conjugation with bioactive molecules and/or as targeting ligands.

Ligand Exchange

Replacement of hydrophobic ligands of NPs with hydrophilic ligands allows the transferring of NPs from organic phase to aqueous solution, which is a prerequisite for biomedical performances. The natural ligand on the surface of NPs can be exchanged by hydrophilic ligands to bring them to the aqueous solution, including small molecules with functional head groups [19, 46].

Innovative Techniques for Bacterial Detection by Using MNPs

The integration of bioconjugate MNPs with different analytical methods has opened a new path for bacteria, protein, and cancer cell sensing, purification, and quantitative analysis. The scope of superparamagnetic nanoparticles at many technological applications like magnetic storage media, biosensing applications, and medical applications made it to develop intensely [47]. In the absence of an external magnetic field, the overall magnetization value of superparamagnetic nanoparticles is randomized to zero. Such fluctuation in magnetization direction results into minimization of the magnetic interactions between any two NPs in the dispersion, making the dispersion stable in physiological solutions and facilitating NPs coupling with biological agents [48]. When exposed to an external magnetic field, these MNPs align along the field of direction, achieving magnetic saturation at a magnitude that far exceeds from any of the known biological entities. Due to this unique property of MNPs, detection of the MNP-containing biological samples is enhanced along with manipulation of these biological samples with an external magnetic field [27].

Recognition Moieties Used for Enrichment of Bacteria

Surface modification of MNPs with recognition moieties such as antibodies, antibiotics (vancomycin, daptomycin, etc.), and carbohydrate enables its use for bacterial detection. These recognition moieties help to detect the bacteria selectively and at low concentration. Different approaches have been used to isolate bacteria using MNPs like as follows.

Use of Antibiotics for Enrichment of Bacteria

Vancomycin belongs to the glycopeptide group of antibiotic, which is known to interact strongly with a broad range of gram-positive bacteria. Vancomycin kills bacteria by inhibiting bacterial cell wall synthesis. This interaction is mediated via five hydrogen bond motifs

between the heptapeptide backbone of vancomycin and the D-alanyl-D-alanine dipeptide from the cell wall [49]. As a result, vancomycin-functionalized MNPs are capable of recognizing the cell surfaces of different bacteria. It has been seen that vancomycin offers less specificity when compared with monoclonal antibody but can bind to different gram-positive bacteria such as *Enterococcus faecalis*, *Streptococcus pneumoniae*, *and Staphylococcus aureus*, but not effective against gram-negative bacteria. Gu et al. reported a strategy to use vancomycin-conjugated FePt magnetic nanoparticles of around 4 nm that are water soluble in nature and used to detect gram-negative bacteria as well as gram-positive bacteria at low concentrations [49, 50]. As a control experiment, they have used FePt nanoparticles capped with amine group ($FePt–NH_2$), which failed to capture the bacteria because of the lack of specific molecular recognition. Lin et al. reported vancomycin-immobilized iron oxide nanoparticles, which can be used to trap gram-positive bacteria such as *Staphylococcus saprophyticus*, *S. aureus*, and *E. faecalis* selectively from urine samples and detected by matrix-assisted laser, desorption/ionization mass spectrometry (MALDI-MS). Their result suggests that this method is capable of rapidly identifying trace pathogens in urine samples [51]. Kell et al. have reported a series of vancomycin-modified Fe_3O_4 MNPs and used in magnetic confinement assay to isolate different gram-positive and gram-negative bacteria at a low concentration. Their results demonstrate that small moieties are an excellent alternative to antibody-mediated detection of bacteria where more precaution is required as compared to small moieties like vancomycin [49]. In 2011, Chung et al. reported that the bio-orthogonal modification of vancomycin and daptomycin, which is lipopeptide in nature and binds to the cell wall of gram-positive bacteria via its hydrophobic tail, resulted in the depolarization of the bacterial cell membrane. Primarily, they have synthesized trans-cyclooctene (TCO) derivatives of this antibiotics, which are attached to tetrazine-decorated Fe_3O_4 fluorescent MNPs. Their result shows that using a two-step labeling procedure, their assay is superior to using direct antibiotic–nanoparticle conjugates [52]. Recently, Chen et al. synthesized fluorescent magnetic nanoparticle with a core shell structure followed by conjugation of gentamycin, which is a FDA-approved thermal-resistant antibiotic belonging to the aminoglycoside group and used for the treatment of infection caused by gram-negative bacteria. Their results demonstrate that gentamicin bioconjugated fluorescent MNPs can capture gram-negative bacteria, i.e., *Escherichia coli* ($1*10^7$ CFU/mL) within 20 min from 10 mL of solution. In addition to this, these gentamycin-modified MNPs are also able to detect diluted *E. coli* cells at a concentration as low as $1*10^3$ CFU/mL [53]. Several such approaches are reported [54–57].

Use of Antibodies for Enrichment of Bacteria

Antibody-conjugated MNPs can selectively capture target bacteria from the given biological sample. Here, application of a magnetic field separates the particle–bacteria complexes from the solution, thereby enriching the concentration of the bacteria and enabling the detection of target bacteria without a culturing process. Use of this approach seems to be more specific in nature. Recently, Quang et al. reported the use of protein A conjugated chitosan-modified Fe_3O_4 MNPs for separation *Vibrio cholerae* at low concentration. In their study, they have prepared a conjugation of chitosan-coated MNPs and protein A. This conjugate was incubated with specific IgG antibodies against *V. cholerae* and can be detected by a conventional diagnostic method as well as immunochromatographic strip test. This method serves as a convenient stage for enrichment and separation of various pathogens from different liquid samples, after just prior incubation with specific IgG antibodies [58]. Several such

immunomagnetic approaches have been developed for the enrichment of MNPs with bacteria and used for detection at a low concentration [59–63].

Use of Other Biomolecules for Enrichment of Bacteria

Biomolecule such as carbohydrate, protein, and nucleic acid is used for enrichment of MNPs. It is known that many bacteria use mammalian cell surface carbohydrates as anchors for attachments, which subsequently results in infection [64]. The unique combination of magnetic nanoparticles and carbohydrate group helps to enrich MNPs with bacteria and can be detected [65]. Pigeon ovalbumin (POA), a phosphoprotein, contains high levels of terminal Gal α(1/ 4) Gal units. Thus, MNPs with immobilized POA can be used as affinity potential probes for bacteria enrichment [66, 67]. Chung et al. have recently developed magneto-DNA nanoparticle system for rapid detection of bacteria. In their work, they have used oligonucleotide probes to detect specifically target nucleic acids particularly 16S rRNAs from the pathogen. Furthermore, the assay is rapid in nature and able to simultaneously detect 13 bacteria specimens within 2 h [68]. A study conducted by Huang et al. has reported the use of amine-functionalized MNPs for capturing of bacteria from water, food, and urine samples. This developed method does not require the use of affinity molecules on the surface and is able to detect different gram-positive and gram-negative bacteria. The detection is based upon the positive charge present on the surface of the MNPs and negatively charged bacterial cell, which promotes a strong electrostatic interaction to exhibit efficient adsorptive ability [69–71].

Figure 3 shows the schematic use of MNPs modified with recognition moieties for enrichment of bacteria followed by detection.

Detection Techniques

There are many instruments, which can be used to detect a range of chemical, optical, and biological signals generated by the aforementioned capture methods. Most detection technologies revolve around measurement of optical, electrochemical, or piezoelectric properties. Gu et al. detected the presence of bacteria by using electron microscopic technique such as SEM and TEM [72]. But it has been seen that the use of the SEM technique is expensive when it comes to clinical settings (Fig. 4). To overcome this difficulty, Gao et al. have used fluorescence for a quick, sensitive, and low-cost technique

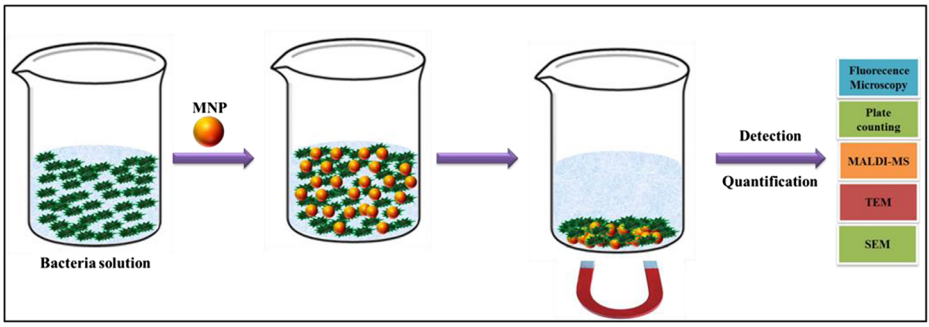

Fig. 3 Schematic representation showing the surface-modified MNPs for detection of pathogens

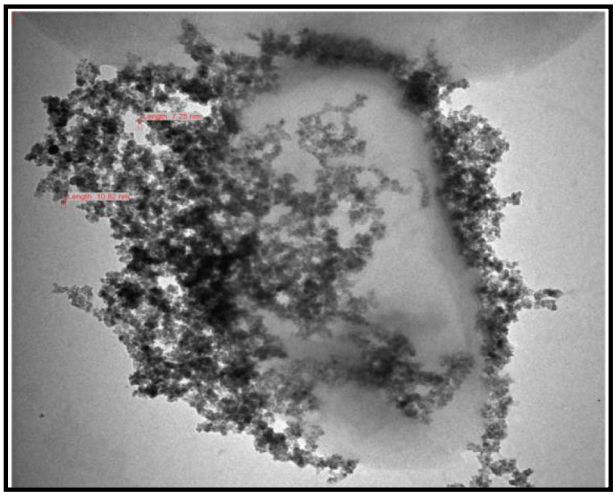

Fig. 4 *E. coli* captured to MNPs [73]

to detect the bacteria. Joo et al. have used anti-salmonella antibody-conjugated MNPs and detected by using TiO_2 nanocrystals. Here, MNPs enabled the rapid extraction of targeted bacteria, and TiO_2 nanocrystals were used as an optical nanoprobe for UV–Visible spectroscopic detection. TiO_2 is stable over a range of pH and less expensive as compared to gold and can be a good alternative for routine detections of bacteria [74]. Cheng et al. have proposed detection of bacteria by using adenosine triphosphate (ATP) bioluminescence method and reported that the proposed method is able to detect bacteria at a concentration of 20 CFU/mL within 1 h [75]. The work by Weissleder's group, who designed an MNP hybridization assay, involves ubiquitous and specific probes of antibiotics that detects gram-positive bacteria by using a nuclear magnetic resonance device [52]. Infrared spectroscopy (IR), and Raman spectroscopies [59, 76] that are known to provide characteristic pathogen-specific fingerprints. Since pathogens differ in various functional groups, IR and Raman spectra can be used as a valuable tool for detection. Wang et al. have reported that characteristic vibrational fingerprint of Raman spectroscopies can be combined with biosensor module to detect multiple bacteria in selected food matrices. The detection limit of their MNP-integrated Raman assay was determined to be 10^3 CFU/mL in spinach solution [76]. Recently, Lee et al. have proposed the detection of pathogenic bacteria using antibody-immobilized magnetic nanoparticle clusters (MNC) and 3D printed helical microchannel. The antibody-immobilized MNC were used to capture *E. coli*, and the solution was injected into helical microchannel with or without sheath flow, and the limit of detection was 100 CFU/mL in milk [63]. Similarly, Wan et al. designed a chemical nose technique for detection of bacteria. Here, q-MNP-fluorescent polymer system is combined to differentiate bacterial cells according to their characteristic response pattern [77]. Chu et al. have reported that a new technique, giant magnetoresistance (GMR), can be used for bacterial detection, where MNPs are tethered to the surface of GMR sensor thin film and change in electric resistance in the sensor is measured [78].

Different techniques used for bacterial detection by employing MNPs and detection limit are summarized in a concise manner in Table 1.

Table 1 Summary of bacterial detection methods

Bacteria	Method of detection	Detection limit
E. coli	MALDI [66]	$9.60*10^4$ CFU/mL
	TEM [71] [72]	NM, 15 CFU/mL
	SEM [72]	15 CFU/mL
	Plate counting [49] [69] [70]	15 CFU/mL
	IR [59]	NM
	FM [65] [54]	10^4–10^5 CFU/mL
	3D microchannel device [63]	10^4 CFU/mL, 110 CFU/mL
		100 CFU/mL
S. aureus	TEM [53]	$0.5*10^3$ CFU/mL
	UV spectrometer [79]	$5*10^1$ CFU/mL
	CFM [80]	10^2 CFU/mL
V. cholera	TEM [58]	10 CFU/mL
B. subtilis	Plate counting [70]	NM
S. lutea	Plate counting [69]	NM
Salmonella complex	UV spectrometer [74]	100 CFU/mL
	3D immunomagnetic flow assay [62]	10 CFU/mL

MALDI-MS matrix-assisted laser desorption ionization mass spectrometry, *CFM* confocal microscope, *FM* fluorescences microscope, *OM* optical microscope, *SEM* scanning electron microscope, *TEM* transmission electron microscope, *NM* not mentioned

Future Outlook

Although functionalized MNPs have great potential applications for bacterial detection, it can be difficult to detect in real-life samples when bacterial concentration is low. In addition, an attempt needs to be made to detect in the presence of other genus and contaminants. Optimum surface modification of nanoparticles is hard to be controlled. Hence, more consistent strategies have to be developed for a precise composition and a uniform surface modification with reproducible functionalization. It is also seen that many works are being carried on Fe_3O_4 MNPs. Other ferrites should be explored in this direction. Future research should be more on sensitivity, reproducibility, and ability of technique, so that it can be used for daily samples.

Conclusion

When functionalized magnetic nanomaterials are tagged with biological ligand, they possess great potential for their applications in the detection of pathogens. Due to their size-dependent physical properties and nanometer-scale dimension, MNPs are shown to be attractive for selective bacteria capture and detection. Properly chosen combinations of functional MNPs allow us to develop multifunctional nanomaterial for fast bacterial detection and removal. This approach provides an attractive and innovative avenue for pathogen diagnostic applications. It is also seen that a single small-molecule-modified nanoparticle can be utilized to target and isolate many different pathogens, preventing the need to prepare specific nanoparticles to target and isolate specific pathogens. As we and several other groups are continuing to explore

this technology, we believe that it will likely lead to the development of exciting techniques or powerful combinations with existing ones for early and rapid detection of bacteria from the sample.

References

1. Newman, E.B. (1994). General microbiology. *Research in Microbiology, 2508*(94), 90009–4.
2. Gracias, K.S., & McKillip, J.L. (2004). A review of conventional detection and enumeration methods for pathogenic bacteria in food. *Canadian Journal of Microbiology, 50*(11), 883–890.
3. Jain, K.K. (2005). Nanotechnology in clinical laboratory diagnostics. *Clinica Chimica Acta, 358*, 37–54.
4. Muthukumar, A., Zitterkopf, N.L., & Payne, D. (2008). Molecular tools for the detection and characterization of bacterial infections: a review. *Laboratory Medicine, 39*(7), 430–436.
5. Sapsford, K.E., Algar, W.R., Berti, L., Gemmill, K.B., Casey, B.J., Oh, E., & Medintz, I.L. (2013). Functionalizing nanoparticles with biological molecules: developing chemistries that facilitate nanotechnology. *Chemical Reviews, 113*(3), 1904–2074.
6. Subbiah, R., Veerapandian, M., & Yun, K.S. (2010). Nanoparticles: functionalization and multifunctional applications in biomedical sciences. *Current Medicinal Chemistry, 17*, 4559–4577.
7. Sapsford, K.E., Tyner, K.M., Dair, B.J., Deschamps, J.R., & Medintz, I.L. (2011). Analyzing nanomaterial bioconjugates: a review of current and emerging purification and characterization techniques. *Analytical Chemistry, 83*, 4453–4488.
8. Jain, K.K. (2007). Applications of nanobiotechnology in clinical diagnostics. *Clinical Chemistry, 53*(11), 2002–2009.
9. Heo, J., & Hua, S.Z. (2009). An overview of recent strategies in pathogen sensing. *Sensors (Switzerland), 9*(6), 4483–4502.
10. Bayer, M.E., & Sloyer, J.L. (1990). The electrophoretic mobility of gram-negative and gram-positive bacteria: an electrokinetic analysis. *Journal of General Microbiology, 136*, 867–874.
11. Nazzaro, F., Fratianni, F., De Martino, L., Coppola, R., & De Feo, V. (2013). Effect of essential oils on pathogenic bacteria. *Pharmaceuticals (Basel, Switzerland), 6*, 1451–74.
12. Zourob, M., Elwary, S., Turner, A. (2008). *Principles of bacterial detection: biosensors recognition receptors and microsystems*. New York: Springer.
13. Niemirowicz, K., Markiewicz, K.H., Wilczewska, A.Z., & Car, H. (2012). Magnetic nanoparticles as new diagnostic tools in medicine. *Advances in Medical Sciences, 57*(2), 196–207.
14. Salunkhe, A.B..., Khot, V.M., & Pawar, S.H. (2014). Magnetic hyperthermia with magnetic nanoparticles: a status review. *Current Topics in Medicinal Chemistry, 14*(5), 572–94.
15. Tartaj, P., Morales, M.A.D.P., Veintemillas-Verdaguer, S., Gonzlez-Carreo, T., & Serna, C.J. (2003). The preparation of magnetic nanoparticles for applications in biomedicine. *Journal of Physics D: Applied Physics, 36*(13), R182–R197.
16. Yallapu, M.M., Othman, S.F., Curtis, E.T., Gupta, B.K., Jaggi, M., & Chauhan, S.C. (2011). Multi-functional magnetic nanoparticles for magnetic resonance imaging and cancer therapy. *Biomaterials, 32*, 1890–1905.
17. Gupta, A.K., & Curtis, A.S. (2004). Surface modified superparamagnetic nanoparticles for drug delivery: interaction studies with human fibroblasts in culture. *Journal of Materials Science Materials in Medicine, 15*, 493–496.
18. Nikam, D.S., Jadhav, S.V., Khot, V.M., Phadatare, M.R., & Pawar, S.H. (2014). Study of AC magnetic heating characteristics of Co0.5Zn0.5Fe2O4 nanoparticles for magnetic hyperthermia therapy. *Journal of Magnetism and Magnetic Materials, 349*, 208–213.
19. Patil, R.M., Shete, P.B., Thorat, N.D., Otari, S.V., Barick, K.C., Prasad, A., Ningyhojam, R.S., Tiwale, B.M., & Pawar, S.H. (2014). Non-aqueous to aqueous phase transfer of oleic acid coated iron oxide nanoparticles for hyperthermia application. *RSC Advances, 4*(9), 4515.
20. Cai, W., & Wan, J. (2007). Facile synthesis of superparamagnetic magnetite nanoparticles in liquid polyols. *Journal of Colloid and Interface Science, 305*(2), 366–70.
21. Zhang, S., Lee, J., & Sun, S. (2012). Controlled synthesis of monodisperse magnetic nanoparticles in solution phase. *The Open Surface Science Journal, 4*(1), 26–34.
22. Bohara, R.A., Thorat, N.D., Yadav, H.M., & Pawar, S.H. (2014). One-step synthesis of uniform and biocompatible amine functionalized cobalt ferrite nanoparticles: a potential carrier for biomedical applications. *New Journal of Chemistry, 38*(7), 2979.

23. Salunkhe, A.B..., Khot, V.M., Thorat, N.D., Phadatare, M.R., Sathish, C.I., Dhawale, D.S., & Pawar, S.H. (2013). Polyvinyl alcohol functionalized cobalt ferrite nanoparticles for biomedical applications. *Applied Surface Science, 264*(3), 598–604.
24. Thorat, N.D., Otari, S.V., Bohara, R.A., Yadav, H.M., Khot, V.M., Salunkhe, A.B..., Phdatre, M.R., Prasad, A.I., Ningthojam, R.S., & Pawar, S.H. (2014). Structured superparamagnetic nanoparticles for high performance mediator of magnetic fluid hyperthermia: synthesis, colloidal stability and biocompatibility evaluation. *Materials Science and Engineering C: Materials for Biological Applications, 42*, 637–46.
25. Wang, L., Luo, J., Shan, S., Crew, E., Yin, J., & Zhong, C.J. (2011). Bacterial inactivation using silver-coated magnetic nanoparticles as functional antimicrobial agents. *Analytical Chemistry, 83*, 8688–8695.
26. Iravani, S. (2011). Green synthesis of metal nanoparticles using plants. *Green Chemistry, 13*, 2638–2650.
27. Xu, C., & Sun, S. (2013). New forms of superparamagnetic nanoparticles for biomedical applications. *Advanced Drug Delivery Reviews, 65*(5), 732–43.
28. Phadatare, M.R., Khot, V.M., Salunkhe, A.B..., Thorat, N.D., & Pawar, S.H. (2012). Studies on polyethylene glycol coating on NiFe2O4 nanoparticles for biomedical applications. *Journal of Magnetism and Magnetic Materials, 324*(5), 770–772.
29. Robinson, I., Tung, L.D., Maenosono, S., Wälti, C., & Thanh, N.T.K. (2010). Synthesis of core-shell gold coated magnetic nanoparticles and their interaction with thiolated DNA. *Nanoscale, 2*, 2624–2630.
30. Yuan, Q., Venkatasubramanian, R., Hein, S., & Misra, R.D.K. (2008). A stimulus-responsive magnetic nanoparticle drug carrier: magnetite encapsulated by chitosan-grafted-copolymer. *Acta Biomaterialia, 4*, 1024–1037.
31. Veiseh, O., Gunn, J.W., & Zhang, M. (2010). Design and fabrication of magnetic nanoparticles for targeted drug delivery and imaging. *Advanced Drug Delivery Reviews, 62*, 284–304.
32. Mohapatra, S., & Pramanik, P. (2009). Synthesis and stability of functionalized iron oxide nanoparticles using organophosphorus coupling agents. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 339*, 35–42.
33. Mamani, J.B., Costa-Filho, A.J., Cornejo, D.R., Vieira, E.D., & Gamarra, L.F. (2013). Synthesis and characterization of magnetite nanoparticles coated with lauric acid. *Materials Characterization, 81*, 28–36.
34. Thorat, N.D., Khot, V.M., Salunkhe, A.B..., Prasad, A.I., Ningthoujam, R.S., & Pawar, S.H. (2013). Surface functionalized LSMO nanoparticles with improved colloidal stability for hyperthermia applications. *Journal of Physics D: Applied Physics, 46*(10), 105003.
35. Hou, C., Zhu, H., Li, Y., Li, Y., Wang, X., Zhu, W., & Zhou, R. (2014). Facile synthesis of oxidic PEG-modified magnetic polydopamine nanospheres for Candida rugosa lipase immobilization. *Applied Microbiology and Biotechnology, 99*(3), 1249–59.
36. Zhu, L., Ma, J., Jia, N., Zhao, Y., & Shen, H. (2009). Chitosan-coated magnetic nanoparticles as carriers of 5-fluorouracil: preparation, characterization and cytotoxicity studies. *Colloids and Surfaces B: Biointerfaces, 68*(1), 1–6.
37. Hu, J., Qian, Y., Wang, X., Liu, T., & Liu, S. (2012). Drug-loaded and superparamagnetic iron oxide nanoparticle surface-embedded amphiphilic block copolymer micelles for integrated chemotherapeutic drug delivery and MR imaging. *Langmuir, 28*, 2073–2082.
38. Reddy, L.H., Arias, J.L., Nicolas, J., & Couvreur, P. (2012). Magnetic nanoparticles: design and characterization, toxicity and biocompatibility, pharmaceutical and biomedical applications. *Chemical Reviews, 112*(11), 5818–78.
39. Mohapatra, S., Rout, S.R., Maiti, S., Maiti, T.K., & Panda, A.B... (2011). Monodisperse mesoporous cobalt ferrite nanoparticles: synthesis and application in targeted delivery of antitumor drugs. *Journal of Materials Chemistry, 21*(25), 9185.
40. Cheng, C., Wen, Y., Xu, X., & Gu, H. (2009). Tunable synthesis of carboxyl-functionalized magnetite nanocrystal clusters with uniform size. *Journal of Materials Chemistry, 19*(46), 8782.
41. Šulek, F., Drofenik, M., Habulin, M., & Knez, Ž. (2010). Surface functionalization of silica-coated magnetic nanoparticles for covalent attachment of cholesterol oxidase. *Journal of Magnetism and Magnetic Materials, 322*, 179–185.
42. Yamaura, M., Camilo, R., Sampaio, L., Macêdo, M., Nakamura, M., & Toma, H. (2004). Preparation and characterization of (3-aminopropyl)triethoxysilane-coated magnetite nanoparticles. *Journal of Magnetism and Magnetic Materials, 279*(2–3), 210–217.
43. Can, K., Ozmen, M., & Ersoz, M. (2009). Immobilization of albumin on aminosilane modified superparamagnetic magnetite nanoparticles and its characterization. *Colloids and Surfaces B: Biointerfaces, 71*(1), 154–9.
44. Patil, R.M., Shete, P.B., Thorat, N.D., Otari, S.V., Barick, K.C., Prasad, A., Ningthoujam, R.S., & Pawar, S.H. (2014). Superparamagnetic iron oxide/chitosan core/shells for hyperthermia application: improved colloidal stability and biocompatibility. *Journal of Magnetism and Magnetic Materials, 355*, 22–30.

45. Shete, P.B., Patil, R.M., Ningthoujam, R.S., Ghosh, S.J., & Pawar, S.H. (2013). Magnetic core–shell structures for magnetic fluid hyperthermia therapy application. *New Journal of Chemistry, 37*(11), 3784.
46. Thorat, N.D., Patil, R.M., Khot, V.M., Salunkhe, A.B..., Prasad, A.I., Barick, K.C., Ningthoujam, R.S., & Pawar, S.H. (2013). Highly water-dispersible surface-functionalized LSMO nanoparticles for magnetic fluid hyperthermia application. *New Journal of Chemistry, 37*(9), 2733.
47. Amstad, E., Textor, M., & Reimhult, E. (2011). Stabilization and functionalization of iron oxide nanoparticles for biomedical applications. *Nanoscale, 3*(7), 2819–43.
48. Ray, P.C., Khan, S.A., Singh, A.K., Senapati, D., & Fan, Z. (2012). Nanomaterials for targeted detection and photothermal killing of bacteria. *Chemical Society Reviews, 41*(8), 3193–209.
49. Kell, A.J., Stewart, G., Ryan, S., Peytavi, R., Boissinot, M., Huletsky, A., Bergeron, M.G., & Simard, B. (2008). Vancomycin-modified nanoparticles for efficient targeting and preconcentration of gram-positive and gram-negative bacteria. *ACS Nano, 2*, 1777–1788.
50. Gu, H., Ho, P.L., Tsang, K.W.T., Wang, L., & Xu, B. (2003). Using biofunctional magnetic nanoparticles to capture vancomycin-resistant enterococci and other gram-positive bacteria at ultralow concentration. *Journal of the American Chemical Society, 125*, 15702–15703.
51. Lin, Y.S., Tsai, P.J., Weng, M.F., & Chen, Y.C. (2005). Affinity capture using vancomycin-bound magnetic nanoparticles for the MALDI-MS analysis of bacteria. *Analytical Chemistry, 77*, 1753–1760.
52. Chung, H.J., Reiner, T., Budin, G., Min, C., Liong, M., Issadore, D., & Weissleder, R. (2011). Ubiquitous detection of gram-positive bacteria with bioorthogonal magnetofluorescent nanoparticles. *ACS Nano, 5*, 8834–8841.
53. Chen, L., Razavi, F.S., Mumin, A., Guo, X., Sham, T.-K., & Zhang, J. (2013). Multifunctional nanoparticles for rapid bacterial capture, detection, and decontamination. *RSC Advances, 3*(7), 2390.
54. Gao, J., Li, L., Ho, P.-L., Mak, G.C., Gu, H., & Xu, B. (2006). Combining fluorescent probes and biofunctional magnetic nanoparticles for rapid detection of bacteria in human blood. *Advanced Materials, 18*(23), 3145–3148.
55. Wan, Y., Zhang, D., & Hou, B. (2010). Determination of sulphate-reducing bacteria based on vancomycin-functionalized magnetic nanoparticles using a modification-free quartz crystal microbalance. *Biosensors and Bioelectronics, 25*, 1847–1850.
56. Gu, H., Xu, K., Xu, C., & Xu, B. (2006). Biofunctional magnetic nanoparticles for protein separation and pathogen detection. *Chemical Communications (Cambridge, England), 9*, 941–9.
57. Chen, L. (2012). Bioconjugated magnetic nanoparticles for rapid capture of gram-positive bacteria. *Journal of Biosensors and Bioelectronics*. doi:10.4172/2155-6210.S11-005. S:11.
58. Tran, Q.H., Pham, V.C., Nguyen, T.T., Blanco Andujar, C., & Thanh, N.T.K. (2014). Protein a conjugated iron oxide nanoparticles for separation of Vibrio cholerae from water samples. *Faraday Discussions, 175*, 73–82.
59. Ravindranath, S.P., Mauer, L.J., Deb-Roy, C., & Irudayaraj, J. (2009). Biofunctionalized magnetic nanoparticle integrated mid-infrared pathogen sensor for food matrixes. *Analytical Chemistry, 81*(8), 2840–2846.
60. Varshney, M., Yang, L., Su, X.-L., & Li, Y. (2005). Magnetic nanoparticle-antibody conjugates for the separation of Escherichia coli O157:H7 in ground beef. *Journal of Food Protection, 68*, 1804–1811.
61. Kumar, V., Nath, G., Kotnala, R.K., Saxena, P.S., & Srivastava, A. (2013). Biofunctional magnetic nanotube probe for recognition and separation of specific bacteria from a mixed culture. *RSC Advances, 3*(34), 14634.
62. Lee, W., Kwon, D., Chung, B., Jung, G.Y., Au, A., Folch, A., & Jeon, S. (2014). Ultrarapid detection of pathogenic bacteria using a 3D immunomagnetic flow assay. *Analytical Chemistry, 86*, 6683–6688.
63. Lee, W., Kwon, D., Choi, W., Jung, G.Y., & Jeon, S. (2015). 3D-printed microfluidic device for the detection of pathogenic bacteria using size-based separation in helical channel with trapezoid cross-section. *Scientific Reports, 5*, 7717.
64. Lingwood, C.A. (1998). Oligosaccharide receptors for bacteria: a view to a kill. *Current Opinion in Chemical Biology, 2*, 695–700.
65. El-boubbou, K., Gruden, C., & Huang, X. (2007). Magnetic glyco-nanoparticles: a unique tool for rapid pathogen detection decontamination, and strain differentiation. *Journal of the American Chemical Society, 129*, 13392–13393.
66. Liu, J.C., Tsai, P.J., Lee, Y.C., & Chen, Y.C. (2008). Affinity capture of uropathogenic Escherichia coli using pigeon ovalbumin-bound Fe3O4@Al2O3 magnetic nanoparticles. *Analytical Chemistry, 80*, 5425–5432.
67. Liu, J.-C., Chen, W.-J., Li, C.-W., Mong, K.-K.T., Tsai, P.-J., Tsai, T.-L., & Chen, Y.-C. (2009). Identification of pseudomonas aeruginosa using functional magnetic nanoparticle-based affinity capture combined with MALDI MS analysis. *The Analyst, 134*, 2087–2094.
68. Chung, H.J., Castro, C.M., Im, H., Lee, H., & Weissleder, R. (2013). A magneto-DNA nanoparticle system for rapid detection and phenotyping of bacteria. *Nature Nanotechnology, 8*(5), 369–75.
69. Huang, Y.-F., Wang, Y.-F., & Yan, X.-P. (2010). Amine-functionalized magnetic nanoparticles for rapid capture and removal of bacterial pathogens. *Environmental Science and Technology, 44*(20), 7908–13.

70. Jin, Y., Liu, F., Shan, C., Tong, M., & Hou, Y. (2014). Efficient bacterial capture with amino acid modified magnetic nanoparticles. *Water Research, 50*, 124–34.
71. Singh, S., Barick, K.C., & Bahadur, D. (2011). Surface engineered magnetic nanoparticles for removal of toxic metal ions and bacterial pathogens. *Journal of Hazardous Materials, 192*(3), 1539–1547.
72. Gu, H., Ho, P., Tsang, W.T., Yu, C., & Xu, B. (2003). Using biofunctional magnetic nanoparticles to capture gram-negative bacteria at an ultra-low concentration †, 1966–1967.
73. Tamer, U., Cetin, D., Suludere, Z., Boyaci, I.H., Temiz, H.T., Yegenoglu, H., Daniel, P., Dincer, I., & Elerman, Y. (2013). Gold-coated iron composite nanospheres targeted the detection of Escherichia coli. *International Journal of Molecular Sciences, 14*, 6223–6240.
74. Joo, J., Yim, C., Kwon, D., Lee, J., Shin, H.H., Cha, H.J., & Jeon, S. (2012). A facile and sensitive detection of pathogenic bacteria using magnetic nanoparticles and optical nanocrystal probes. *The Analyst, 137*(16), 3609–12.
75. Cheng, Y., Liu, Y., Huang, J., Li, K., Zhang, W., Xian, Y., & Jin, L. (2009). Combining biofunctional magnetic nanoparticles and ATP bioluminescence for rapid detection of Escherichia coli. *Talanta, 77*(4), 1332–1336.
76. Wang, Y., Ravindranath, S., & Irudayaraj, J. (2011). Separation and detection of multiple pathogens in a food matrix by magnetic SERS nanoprobes. *Analytical and Bioanalytical Chemistry, 399*, 1271–1278.
77. Wan, Y., Sun, Y., Qi, P., Wang, P., & Zhang, D. (2014). Quaternized magnetic nanoparticles-fluorescent polymer system for detection and identification of bacteria. *Biosensors and Bioelectronics, 55*, 289–293.
78. Chu, Y.W., Engebretson, D.A., & Carey, J.R. (2013). Biconjugated magnetic nanoparticles for the detection of bacteria. *Journal of Biomedical Nanotechnology, 9*(12), 1951–1961.
79. Chen, X., Wu, X., Gan, M., Xu, F., He, L., Yang, D., Xu, H., Shah, N.P., & Wei, H. (2015). Rapid detection of Staphylococcus aureus in dairy and meat foods by combination of capture with silica-coated magnetic nanoparticles and thermophilic helicase-dependent isothermal amplification. *Journal of Dairy Science, 98*, 1563–1570.
80. Bhattacharya, D., Chakraborty, S.P., Pramanik, A., Baksi, A., Roy, S., Maiti, T., Ghosh, S.K., & Pramanik, P. (2011). Detection of total count of Staphylococcus aureus using anti-toxin antibody labelled gold magnetite nanocomposites: a novel tool for capture, detection and bacterial separation. *Journal of Materials Chemistry, 21*(43), 17273–17282.