Published in final edited form as:

Nanomedicine (Lond). 2011 October ; 6(8): 1295–1296. doi:10.2217/nnm.11.129.

Nanoparticles for Rapid Detection of Microbial Threats

Xiaoning Li and Vincent M. Rotello*

Vincent M. Rotello: rotello@chem.umass.edu

*Department of Chemistry, University of Massachusetts, 710 North Pleasant Street, Amherst, Massachusetts 01003

Keywords

nanoparticles; microbial detection; biosensors

The *E. coli* outbreak in Germany and the NDM-expressing “super bugs” in Asia remind us that microorganisms are one of the biggest threats to the public health on the global scale. As a result, rapid and sensitive detection of microbes is a key requirement in food safety, environmental monitoring, and clinical diagnosis and treatment. Conventional techniques for pathogenic bacteria identification and detection include the culture and colony counts,¹ polymerase chain reaction (PCR),² and immunological approaches.³ These classic methods, though powerful and reliable, are slow (usually taking more than 24 hours), generating a critical delay for environmental monitoring and diagnoses. Additionally, these methods are expensive to implement and require trained personnel, a particularly severe limitation for developing nations.

Advances in nanotechnology provide new tools for the rapid and sensitive microbial detection. Nanoparticles (NPs), for example, feature unique size-dependent properties such as enhanced surface reactivity, surface plasmon resonance, quantum confinement, and superparamagnetism, making them excellent materials for the creation of next generation biosensors.

There are two primary ways to identify bacteria, genotyping and phenotyping. Nanoparticles have been incorporated quartz crystal microbalance (QCM) DNA sensors.⁴ For example, a single-strand DNA probe complementary to the target bacterial DNA was immobilized onto the QCM sensor surface. Upon exposure to the target DNA, hybridization was induced and streptavidin conjugated Fe₃O₄ nanoparticles were then used to bind with biotinylated target DNA as “mass enhancers” to amplify the frequency change. This method could identify 3× 10² *Escherichia coli* (*E.coli*) O157:H7 cells/ml.

Phenotypic identification of bacteria focuses on morphological or biomarker differences between bacteria. Biomarkers on bacterial cell surfaces are more accessible and sensing based on recognition of surface biomarkers eliminates the need of analyte pre-treatment, offering rapid detection. NPs functionalized with bacteria-specific antibodies can specifically bind to one type of bacteria, and various properties of NPs provide different sensing principles. Ravindranath and co-workers used magnetic NPs conjugated with anti-*E. coli* O157:H7 or anti-*Salmonella typhimurium* antibodies that can specifically bind to their

target organisms to isolate *E. coli* O157:H7 and *S. typhimurium* separately from a cocktail of bacteria.⁵ The labeled pathogens were detected later by a portable mid-IR spectrometer that enabled the approach as a point-of-care detection technology with a limit of detection (LOD) 10^4 – 10^5 cells/mL. In an alternate strategy, Lee et al. reported a novel, miniaturized diagnostic magnetic resonance (DMR) system using magnetic particles as a proximity sensor to amplify molecular interactions.⁶ Magnetic NPs functionalized with vancomycin bind to D-alanyl-D-alanine moieties in the bacterial cell wall, causing relaxation time decreases in a concentration-dependant fashion. This highly sensitive system was able to detect 10^3 cells/mL of *Staphylococcus aureus* as a model organism. Electrostatic interaction with bacterial cells has also been utilized for bacterial detection. Positively charged hydroxyl-terminated polyamidoamine (PAMAM) dendrimers were demonstrated to capture bacterial cells via electrostatic interactions during bacteria sensing process. A real time sensor for waterborne bacteria used a film of amine-terminated G₄ PAMAM dendrimers with embedded reporter fluorophores, giving the limit of detection (LOD) of 10^4 cells/mL.⁷

Strategies relying on specific recognition of bacterial surfaces require pre-knowledge of target analytes, with the resulting sensors limited to specific strains of bacteria. Selective interactions with bacteria provide a more general pathway in microbial detection. AuNPs functionalized with quaternary amine ligands provide an excellent system for array-based “chemical nose” sensing approaches.⁸ In one example by Rotello, an array of gold nanoparticle–conjugated fluorescent polymer constructs achieved rapid detection and identification of 9 different species of bacteria as well as 3 strains of *E. coli*.⁹ In this system, cationic gold nanoparticles were bound to poly (paraphenyleneethynylene) (PPE) in solution to afford fluorescence-quenched complexes, with signal generation occurring upon displacement of the polymer from the NP surface by bacteria.

Two major challenges for widespread implementation of bacterial sensors are limits of detection and ease/expense of implementation. To address these issues, Rotello has developed a colorimetric nanocomposite biosensor using an enzymatic amplification process to provide high sensitivity for detection of bacteria.¹⁰ β -Galactosidase (β -Gal) was reversibly bound to cationic AuNPs, inhibiting enzymatic activity. Binding of bacteria to the cationic particle surface displaced the β -Gal with concomitant restoration of activity that was transduced using a chromogenic substrate. This system detected *E. coli* at 10^2 cells/mL. This strategy was applied to a test-strip-based format, providing rapid detection with limits of 10^4 cells/mL.

Taken together, the size and properties of nanoparticles make them excellent platforms for detection and identification of pathogens. Recent studies have incorporated these particles in both devices and “low tech” test strip formats, providing a glimpse of the potential versatility of these systems in microbial sensing. Clearly, there are many directions that can be pursued in the area of pathogen detection, with the genuine potential to save lives and improve the world in myriad other ways.

Acknowledgments

Support of the NIH (GM077173-05) is gratefully acknowledged.

References

1. Allen MJ, Edberg SC, Reasoner DJ. Heterotrophic plate count bacteria - what is their significance in drinking water? *Int J Food Microbiol.* 2004; 92:265–274. [PubMed: 15145585]
2. Burtscher C, Wuertz S. Evaluation of the use of PCR and reverse transcriptase PCR for detection of pathogenic bacteria in biosolids from anaerobic digestors and aerobic composters. *Appl Environ Microbiol.* 2003; 69:4618–4627. [PubMed: 12902250]
3. Van Dyck E, Ieven M, Pattyn S, Van Damme L, Laga M. Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by enzyme immunoassay, culture, and three nucleic acid amplification tests. *J Clin Microbiol.* 2001; 39:1751–1756. [PubMed: 11325985]
4. Mao XL, Yang LJ, Su XL, Li YB. A nanoparticle amplification based quartz crystal microbalance DNA sensor for detection of *Escherichia coli* O157 : H7. *Biosens Bioelectron.* 2006; 21:1178–1185. [PubMed: 15951163]
5. Ravindranath SP, Mauer LJ, Deb-Roy C, Irudayaraj J. Biofunctionalized magnetic nanoparticle integrated mid-infrared pathogen sensor for food matrixes. *Anal Chem.* 2009; 81:2840–2846. [PubMed: 19281189]
6. Lee H, Sun E, Ham D, Weissleder R. Chip-NMR biosensor for detection and molecular analysis of cells. *Nat Med.* 2008; 14:869–874. [PubMed: 18607350]
7. Ji J, Schanzle A, Tabacco MB. Real-time detection of bacterial contamination in dynamic aqueous environments using optical sensors. *Anal Chem.* 2004; 76:1411–1418. [PubMed: 14987098]
8. You CC, Miranda OR, Rotello VM, et al. Detection and identification of proteins using nanoparticle-fluorescent polymer 'chemical nose' sensors. *Nat Nanotech.* 2007; 2:318–323.
9. Phillips RL, Miranda OR, You CC, Rotello VM, Bunz UHF. Rapid and efficient identification of bacteria using gold-nanoparticle - Poly(para-phenyleneethynylene) constructs. *Angew Chem Int Ed.* 2008; 47:2590–2594.
10. Miranda OR, Li X, Rotello VM, Bunz UHF, et al. Colorimetric bacteria sensing using a supramolecular enzyme-nanoparticle biosensor. *J Am Chem Soc.* 2011; 133:9650–9653. [PubMed: 21627131]