An integrated NMR/nanosensor system for sensitive detection of environmental toxins and harmful microbes

J. Manuel Perez and Charalambos Kaittanis

Nanoscience Technology Center, Department of Chemistry and Biomolecular Science Center
12424 Research Parkway, Suite 400, Orlando, FL 32826, jmperez@mail.ucf.edu

ABSTRACT

The use of magnetic nanoparticles in conjunction with NMR detection technologies has lead to significant improvements in the detection of various molecular targets with high sensitivity and selectivivity in complex media. Recently, superparamagnetic iron oxide nanosensors have been designed to quantify various biomolecular targets, demonstrating high sensitivity and specificity [1]. Using this technique various targets such as nucleic acids (DNA and mRNA), proteins and even viruses have been detected, with a sensitivity in the low femtomole range (0.5 – 30 fmol) for DNA. The observed changes in T2 are directly proportional to the concentration of the target in solution and can be easily detected by existing magnetic resonance (NMR/MRI) techniques. In this report, we present recent work geared towards the detection of pathogens and toxins.

Keywords: pathogens, toxins, sensing, iron oxide, magnetic nanoparticles

1 INTRODUCTION

The principle underlying the detection mechanism of these magnetic relaxation nanosensors is based on their ability to switch between a dispersed and clustered (or assembled) state upon target interaction, with a concomitant change in the spin-spin relaxation time (T2) of the solution's water protons (Figure 1). This NMR-based detection approach requires no separation; it is robust to interferences and has been performed in whole blood, lipid emulsion, and tissue culture media. These magnetic nanosensors are composed of a 4-8 nm core of superparamagnetic iron oxide core surrounded by a crosslinked dextran coating, resulting in a nanoparticle of 30-40 nm in size. In order to enhance their stability and functionality, the dextran coating is crosslinked with epichlorohydrin and followed by ammonia treatment to incorporate accessible functional amino groups. These nanoparticles exhibits great stability, even under extremely harsh conditions, such as a 30-minute incubation at 120 °C, with neither size nor coating alterations. Conjugation of biomolecules such as peptides, proteins, antibodies and oligonucleotides to the nanoparticles, using various crosslinking agents and conjugation chemistries, creates target specific nanosensors that have been utilized in the detection of various targets.

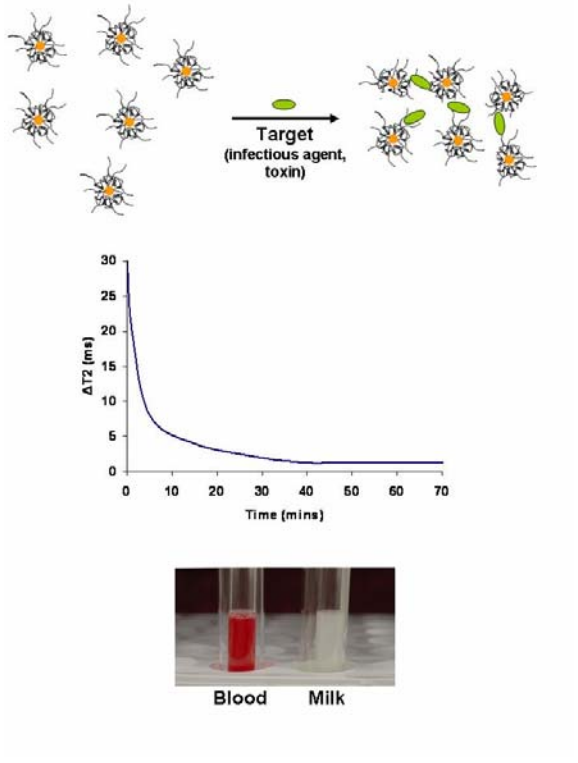

Figure 1. Magnetic nanosensor principle. The detection of the target via magnetic relaxation is fast and can be done in opaque media.

In particular, pathogen- and toxin-specific nanosensors can be designed by conjugating the corresponding antibody on the nanoparticle via Protein G. Additionally, fluorescent dyes have been conjugated to the magnetic nanosensors allowing for multimodal (magnetic and optical) sensing.

2 VIRAL SENSING

One of the first applications of the use of these magnetic nanosensors for the identification of pathogens, was the sensing and quantification of viral particles in serum [2]. It was hypothesized that the presence of multiple copies of a viral protein on the viral coat facilitates multivalent

interactions between the multi-epitope virions and the magnetic nanosensors. This feature would enhance nanoassembly formation; therefore, promoting high sensitivity for viral detection. Having this in mind, magnetic nanoparticles were designed to sense a specific virus, such as herpes simplex virus-1 (HSV-1) and adenovirus-5 (ADV-5). Using these viral-sensing magnetic nanosensors, low levels of virus (5 virions per 10 μL) were detected in serum. The identification of viral particles with magnetic nanosensors outperforms the detection of viruses with contemporary PCR techniques, providing quick and easy-to-read results with minimal artifacts, and without the need for protein removal or sample amplification. In the future, acknowledging the potential of this assay, viral-specific nanoparticles can be designed for the detection and determination of the localization of viruses *in vivo*, serving as viral-specific MRI agents.

3 BACTERIAL SENSING

Most recently, we have been able to develop bacteria-specific magnetic nanosensors [3]. For these studies, we used *Mycobacterum avium* spp. *paratuberculosis* (MAP), as our model organism. We selected this bacterium because its growth in culture is difficult, slow and its identification with current methods is not easy. Furthermore, this bacteria is known to be present in the blood and milk of cattle and it is known to be responsible for Johne's disease in cattle and presumably Crohn's disease in humans. The development of bacterial-specific nanosensors has allowed the sensing of this specific bacterial target (MAP) in complex media (whole milk and blood) with high specificity and sensitivity. The MAP nanosensors used in this study were prepared by conjugating anti-MAP antibodies to superparamagnetic iron oxide nanoparticles via Protein G. Upon addition of the bacteria, formation of the bacterial-induced nanoassembly was detected via magnetic relaxation measurements almost immediately in both phosphate buffer and milk. The specificity of our nanosensors towards the bacteria (MAP) was tested by comparing the sensors response to various other types of bacteria. Figure 2 shows that the sample that had MAP alone (115 Colony Forming Units [CFUs] in 10 μL) had the highest change in T2, while the samples that had other bacteria (~10^6 CFUs in 10 μL) demonstrated minimal T2 changes. More importantly, the sample containing a mixture of bacteria, including MAP (77.5 CFUs in 10 μL), was identified as MAP-positive, despite the presence of interference, underlying the specificity of our nanosensors. Detection and quantification of MAP in milk was done by incubating MAP-spiked whole milk with MAP nanosensors (2.1 μg Fe/μL). We found that the change in T2 was indirectly proportional to the MAP concentration, supporting our proposed detection model. Reliable quantification of MAP from 15.5 to 775 CFUs (R^2=0.93) was achieved after a 30-minute incubation at room temperature (Figure 3). In control experiments using nanoparticles with no MAP antibody minimal changes in T2 were observed. This approach, apart from sensitive and fast, is independent of the sample's optical properties, requires minimum sample preparation and can be used at the points-of-care. This method provides a novel approach for microbial detection that can potentially expedite decision making in a broad range of fields including the clinical, environmental and agricultural sectors.

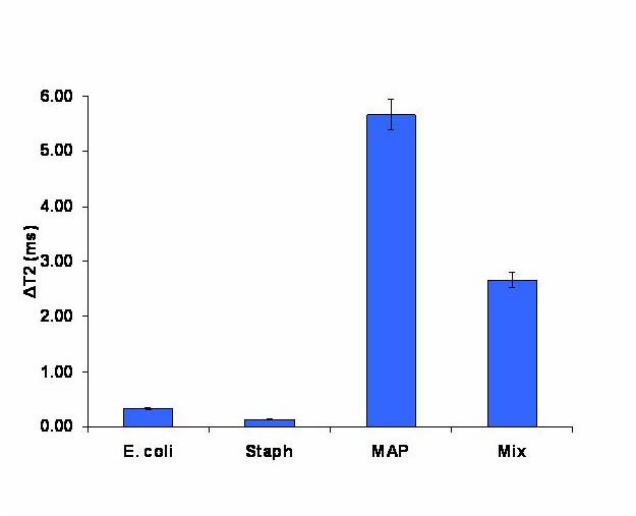

Figure 2. Specificity of the MAP nanosensors

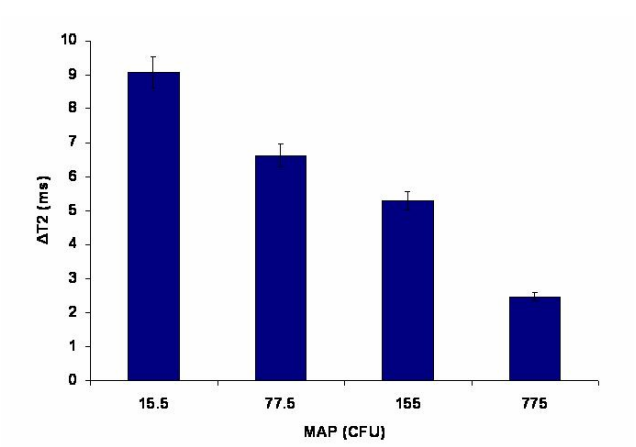

Figure 3. Concentration dependent behavior of MAP magnetic nanosensors.

4 POTENTIAL FOR SENSING TOXINS

The fact that the developed magnetic nanosensors can facilitate rapid detection of a molecular target without extensive sample preparation or target amplification in turbid samples and cam work in the presence of

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interferences makes them attractive for developing sensing technologies to monitor the presence of toxins and pathogens in environmental and clinical samples. Complex and opaque media such as blood, cell suspensions, culture media, lipid emulsions and even whole tissue can be used. Additionally, there is no need for sample immobilization onto a flat surface, such as in the case of microarrays, facilitating faster hybridization and monitoring of binding kinetics. Current work is geared toward developing technologies for the detection of ricin toxin, anthrax, and other highly pathogenic bacteria, among others applications.

5 MAGNETIC RELAXATION DETECTORS

Using high-throughput NMR, hundreds of environmental samples can be screened, drastically speeding up the screening of a library of nanoparticles for detecting a particular target (toxin or pathogen). In addition, high-throughput NMR can be used to screen multiple samples collected at various locations for the presence of a particular target in a matter of hours. It would be difficult to implement a portable and deployable system for environmental sensing and point-of-care diagnostics that required a bulky detection system (NMR or MRI). Since spectroscopic or 3D information is not required to measure the target-induced changes in magnetic relaxation, a simple magnetic relaxometer (0.47 T Bruker MiniSpec) will be sufficient. In fact, most of the work published in the literature has been done using a 0.47 T Bruker MiniSpec. However, a briefcase-sized NMR relaxometer would be ideal for this application. To advance this work, we are collaborating with a company to develop such a device and initial testing reveals that it can sense for various toxins with high sensitivity and selectivity in a matter of minutes.

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