Identification of bacterial drug resistance in blood using iron oxide nanoparticles

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ABSTRACT

Development of antibiotic resistance by microorganisms has been on the rise in recent years. Therefore, identifying drug resistant strains and effective antibiotic agents is critical. Traditional microbiological methods assess antimicrobial susceptibility within 24 to 48 hours, whereas optical nanoparticle-based methods cannot be used in opaque media. Hence, we have developed a nanoparticle-based antimicrobial susceptibility assay, utilizing Concanavalin A-conjugated iron oxide nanoparticles. When the bacteria do not grow, the changes in the solution's T2 relaxation times are proximal to those of the sterile medium. On the other hand, when the bacteria grow, the levels of free carbohydrates decrease, thus the changes in the T2 times are significantly higher than those of the sterile medium. The iron-oxide-nanoparticle-based antimicrobial susceptibility assay (i) monitors bacterial metabolism, (ii) provides results within 2 hours, (iii) determines the minimum effective antibiotic concentration with sensitivity comparable to those of the gold standard methods, and (iv) determines antimicrobial susceptibility in biological fluids, such as blood.

Keywords: antimicrobial susceptibility, iron oxide nanoparticles, bacteria

Within the last 20 years, there has been a dramatic increase in the incidence of antibiotic-resistant bacteria [1, 2]. Specifically, the emergence of drug resistant strains of *Mycobacterium tuberculosis* and the increasing numbers of methicillin-resistant *Staphylococcus aureus* (MRSA) infections indicate that drug resistance is a major public health problem, causing more mortality to the US than HIV/AIDS[1-3]. Hence, developing robust, sensitive and cost-efficient diagnostic modalities that can quickly determine bacterial drug resistance and effective antibiotic concentrations (minimum inhibitory concentration – MIC) is critical for treatment and the containment of epidemics, as the gold standard method for MIC determination requires 24 - 48 hours in transparent media[4, 5].

Nanotechnology can quickly detect microbial antigens in small sample volumes, with great sensitivity and specificity[6-10]. However, nanoparticle-based immunoassays cannot assess if the pathogen is metabolically active or dead[10, 11]. Recently, we reported a gold nanoparticle-based method for the assessment of MIC via the surface plasmon resonance shifts provided fast and reliable results[12], yet this method cannot be used in turbid or opaque media. Therefore, as some microorganisms can be present in the blood of infected patients (septicemia)[13, 14], we hypothesized that iron oxide nanoparticles may quickly determine bacterial susceptibility in blood via water relaxation changes. Specifically, we reasoned that conjugation of Concanavalin A (ConA), a protein with high affinity to carbohydrates[15,16], to iron oxide nanoparticles would allow complex carbohydrate quantification and MIC determination in blood (**Figure 1**). This was justified by the fact that in the absence of bacterial metabolism the carbohydrate levels of the medium would have been comparable to those of the sterile medium, resulting in small changes in the relaxation times (ΔT2), contrary to the large shifts under active metabolism (**Figure 1**).

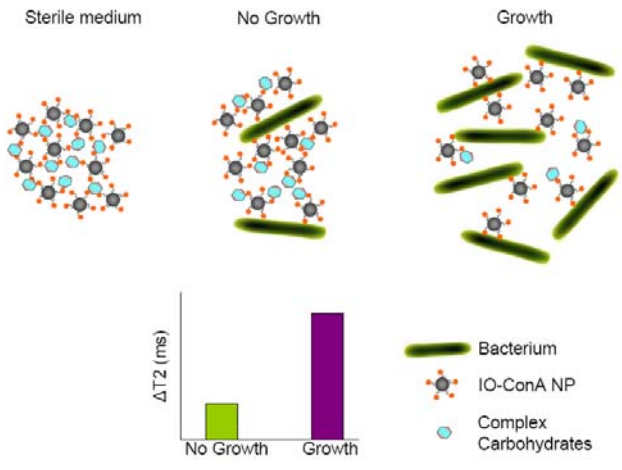

Figure 1. Differential behavior of ConA-conjugated iron oxide nanoparticles in response to bacterial metabolism.

For our studies, we synthesized silica-coated iron oxide nanoparticles and conjugated ConA to them via EDC chemistry. We determined that their diameter was 145 nm and their R2 relaxivity was 225 $mM^{-1}s^{-1}$. Subsequently, we used these nanosensors to assess the antimicrobial susceptibility of two microorganisms in blood. *E. coli* (antibiotic sensitive) and *S. marcescens* (antibiotic resistant) were incubated with various concentrations of ampicillin in blood, at 37°C for 2 hours. Upon addition of the 10-μL sample to the nanosensor solution distinct changes were observed (**Figure 2**). Notably, the nanosensors determined that *E. coli* was susceptible to ampicillin, exhibiting an MIC of 8 μg (**Figure 2**). Furthermore, we were able to determine that *S. marcescens* was resistant to ampicillin, as there were no statistical differences between the sample without ampicillin and the ones that had it (**Figure 2**). Also, the nanosensors did not precipitate when they were incubated with the blood sample, demonstrating the assay's robustness in complex biological media (**Figure 2**). We corroborated our results with the turbidity method, as it is the gold standard assay for MIC determination. However, as this method cannot provide results in blood, we determined the microorganisms' antimicrobial susceptibility in transparent media (MH broth). The turbidity method provided results within 24 hours, demonstrating that for *E. coli* the MIC was indeed 8 μg of ampicillin (**Figure 2**).

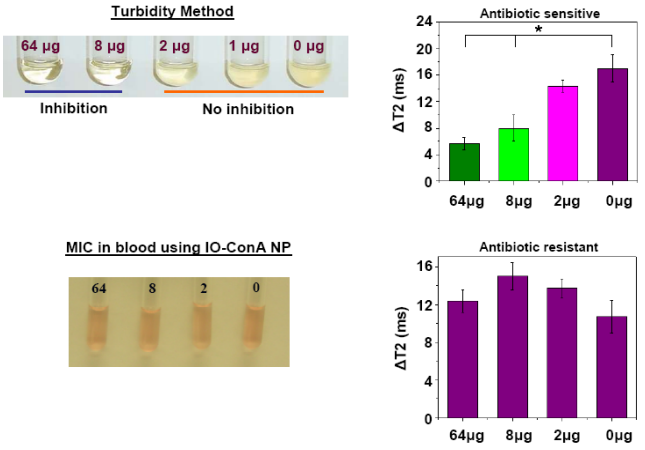

Figure 2. Assessment of antimicrobial susceptibility through the turbidity method in MH broth and the T2 changes in blood using the iron oxide-ConA nanoparticles, requiring 24 and 2 hours respectively.

In addition to these studies, we have used the IO-ConA nanosensors for the monitoring of bacterial metabolic activity and antibiotic susceptibility of fastidious microorganisms, such as *Shigella sonnie*. Concluding, the complex-carbohydrate-sensing metabolism-monitoring IO-ConA can be used for antimicrobial susceptibility assays, yielding reliable and faster results, while utilizing readily available instrumentation and minute sample volumes. Considering these, we anticipate that these iron oxide nanosensors can be used in the clinic and the field, expediting clinical decision-making in point-of-care diagnostics, quickly identifying bacterial resistant stains, rapidly determining effective antibiotics during epidemics and promoting the discovery and development of new antimicrobial agents in complex biological media.

REFERENCES

[1] Bancroft, E. A. *Jama* **2007,** 298, (15), 1803-4.
[2] Klevens, R. M.; Morrison, M. A.; Nadle, J.; Petit, S.; Gershman, K.; Ray, S.; Harrison, L. H.; Lynfield, R.; Dumyati, G.; Townes, J. M.; Craig, A. S.; Zell, E. R.; Fosheim, G. E.; McDougal, L. K.; Carey, R. B.; Fridkin, S. K. *Jama* **2007,** 298, (15), 1763-71.
[3] Liu, C. I.; Liu, G. Y.; Song, Y.; Yin, F.; Hensler, M. E.; Jeng, W. Y.; Nizet, V.; Wang, A. H.; Oldfield, E. *Science* **2008,** 319, (5868), 1391-4.
[4] Murray, P. R.; Baron, E. J., *Manual of clinical microbiology*. 9th ed.; ASM Press: Washington, D.C., 2007.
[5] Andrews, J. M. *J Antimicrob Chemother* **2001,** 48 Suppl 1, 5-16.
[6] Alivisatos, A. P.; Gu, W.; Larabell, C. *Annu Rev Biomed Eng* **2005,** 7, 55-76.
[7] Bruchez, M., Jr.; Moronne, M.; Gin, P.; Weiss, S.; Alivisatos, A. P. *Science* **1998,** 281, (5385), 2013-6.
[8] Ferrari, M. *Nat Rev Cancer* **2005,** 5, (3), 161-71.
[9] Rosi, N. L.; Mirkin, C. A. *Chem Rev* **2005,** 105, (4), 1547-62.
[10] Kaittanis, C.; Naser, S. A.; Perez, J. M. *Nano Lett* **2007,** 7, (2), 380-3.
[11] Phillips, R. L.; Miranda, O. R.; You, C. C.; Rotello, V. M.; Bunz, U. H. *Angew Chem Int Ed Engl* **2008,** 47, (14), 2590-4.
[12] Nath, S.; Kaittanis, C.; Tinkham, A.; Perez, J. M. *Anal Chem* **2008,** 80, (4), 1033-8.
[13] Katneni, R.; Hedayati, S. S. *Nat Clin Pract Nephrol* **2007,** 3, (5), 256-66.
[14] Tapsall, J. W. *Clin Infect Dis* **2005,** 41 Suppl 4, S263-8.
[15] Aslan, K.; Lakowicz, J. R.; Geddes, C. D. *Anal Biochem* **2004,** 330, (1), 145-55.
[16] Yoshizumi, A.; Kanayama, N.; Maehara, Y.; Ide, M.; Kitano, H. *Langmuir* **1999,** 15, (2), 482-488.