Identification of bacterial susceptibility to antibiotics using gold nanoparticles

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ABSTRACT

Inexpensive and rapid bacterial detection and antimicrobial susceptibility assays are desperately needed, as the determination of which antibiotic to use and its administration at effective dosages is critical. Currently, the assessment of antimicrobial susceptibility takes 24 to 48 hours. Hence, we have developed a nanoparticle-based antimicrobial susceptibility assay, utilizing the Concanavalin A-induced clustering of dextran-coated gold nanoparticles. When the bacteria do not grow, addition of Concanavalin A promotes the formation of extensive dextran gold nanoassemblies. The induction of these nanoassemblies is mediated by the presence of free carbohydrates, causing large shifts in the nanoparticles' surface plasmon band. In contrast, when the bacteria grow, the levels of free carbohydrates decrease, thus the size of the gold nanoparticle clusters and the plasmonic shifts are smaller. This gold nanoparticle-based assay provides results within 3 hours and can be used for the high-throughput screening of samples during epidemics.

Keywords: antimicrobial susceptibility, gold nanoparticles, bacteria

Quick determination and administration of the most effective antibiotic is critical for treatment of bacterial infections[1]. Specifically, the rise of multi-drug resistant bacterial strains highlights the need for fast and accurate bacterial identification and the corresponding expedite determination of susceptibility to antibiotics (antimicrobial susceptibility). Currently, antimicrobial susceptibility assays depend on the isolation of the microorganism and culture examination of its growth in the presence of various antibiotic agents[2]. The major limitation of this approach is that it needs more than 24 hours to provide conclusive results, and cannot be utilized in a high-throughput fashion. Although nanoparticle-based sensors have been developed for the specific and sensitive detection of enzymatic activity[3-6], small molecules[7, 8], nucleic acids[6, 8-14], proteins[6, 8, 10, 15, 16], and even whole organisms such as viruses and bacteria[8, 17-20], nanotechnology-based assays have not been designed for the rapid determination of the determination of antimicrobial susceptibility.

Considering the above, we have designed an antimicrobial susceptibility assay, using the clustering of dextran-coated gold nanoparticles upon addition of Concanavalin A (Con A). This allows the fast, inexpensive and high-throughput determination of antibiotic efficacy and their effective dosages. Specifically, we hypothesized that in the presence of an effective antibiotic, the bacteria will not proliferate. Hence upon addition of Con A, we would observe extensive nanoparticle clustering and plasmonic shifts similar to those of the sterile medium (**Figure 1**). On the other hand, in the absence of an effective antibiotic, the bacteria will proliferate, consuming large amounts of the medium's polysaccharides. As a result, addition of Con A will mediate the formation of smaller nanoassemblies, accompanied with greater plsasmonic shifts (Δλ) compared to the sterile control (**Figure 1**).

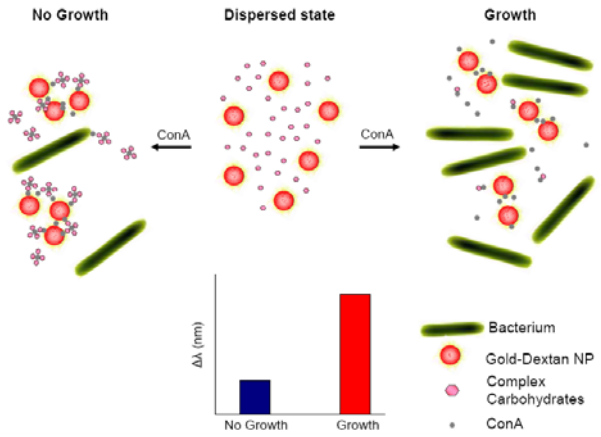

Figure 1. Differential behavior of dextran-coated gold nanoparticles in response to bacterial metabolism.

After synthesizing our dextran-coated gold nanoparticles, we determined that their diameter was ~25 nm, through

TEM and DLS. Subsequent UV-vis analysis of these nanoparticles, in either buffer or sterile medium, demonstrated an absorption maximum at 531 nm (**Figure 2**). This maximum was not affected by the presence of bacteria grown under inhibitory (64 μg ampicillin) or non-inhibitory conditions (2 μg ampicillin), suggesting that possible changes upon Con A-induced clustering might induce different absorption spectra (**Figure 2**). Treatment with Con A (10 $\mu g ml^{-1}$) facilitated rapid plasmonic shifts in all samples. Specifically, after 30 minutes, the sample containing sterile medium exhibited the highest change, followed by the one where growth was suppressed (inhibitory antibiotic concentration). Specifically, the $\Delta\lambda$ of the latter sample was only 2 nm, whereas that of the sample where bacteria proliferated (non-inhibitory antibiotic concentration) was 9 nm (**Figure 2**).

Interestingly, through DLS studies, we assessed that in both the sterile medium and the growth suppression sample large nanoassemblies were formed upon Con A addition (60% of clusters greater than 350 nm and 40% smaller than 100 nm). Contrary, the sample with proliferating bacteria had smaller nanoclusters (40% were greater than 350 nm and 60% smaller than 100 nm), further corroborating the observed plasmonic shifts. We confirmed the nanoparticle-based antimicrobial susceptibility results through the turbidity method, which is the gold-standard assay for the determination of antimicrobial susceptibility. After 24 hours, identical results were obtained, although the gold-nanoparticle-based assay yielded results within only 3 hours (**Figure 2**). Lastly, we simultaneously screened numerous samples and determined antimicrobial susceptibility in a high-throughput format, using a microtiter plate reader (**Figure 2**).

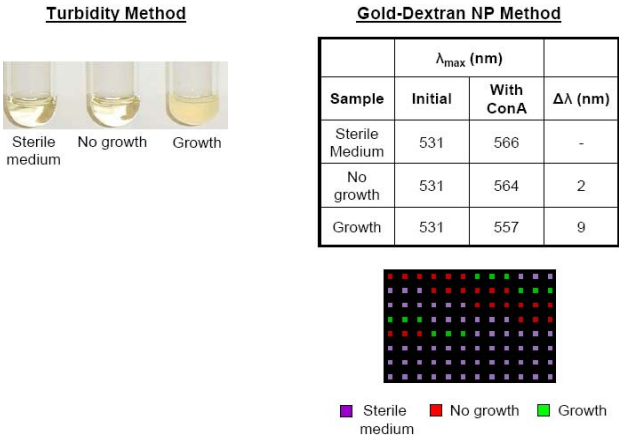

Figure 2. Determination of antimicrobial susceptibility through the turbidity method and via the plasmonic shifts of gold nanoparticles, requiring 24 and 3 hours respectively.

Concluding, the developed gold nanoparticle-based antimicrobial susceptibility assay provides reliable and faster results, utilizing readily available instrumentation and requiring minuscule sample volumes. Based on these traits, we anticipate that the gold nanoparticle-based assay can promote faster clinical decision-making in point-of-care diagnostics, and may be highly utilized for the high-throughput screening of samples during epidemics and discovery of new antimicrobial agents.

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