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Interactions between bacterial surface and nanoparticles govern the performance of "chemical nose" biosensors

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ABSTRACT

Rapid and portable diagnosis of pathogenic bacteria can save lives lost from infectious diseases. Biosensors based on a "chemical nose" approach are attracting interest because they are versatile but the governing interactions between bacteria and the biosensors are poorly understood. Here, we use a "chemical nose" biosensor based on gold nanoparticles to explore the role of extracellular polymeric substances in bacteria-nanoparticle interactions. We employ simulations using Maxwell-Garnett theory to show how the type and extent of aggregation of nanoparticles influence their colorimetric response to bacteria. Using eight different species of Gram-positive and Gram-negative bacteria, we demonstrate that this "chemical nose" can detect and identify bacteria over two orders of magnitude of concentration (89% accuracy). Additionally, the "chemical nose" differentiates between binary and tertiary mixtures of the three most common hospital-isolated pathogens: *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* (100% accuracy). We demonstrate that the complex interactions between nanoparticles and bacterial surface determine the colorimetric response of gold nanoparticles and thus, govern the performance of "chemical nose" biosensors.

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1. Introduction

Conventional biosensors focus on a 'lock and key' recognition strategy (Rotello, 2009), which utilizes biomolecules such as aptamers and antibodies to offer high sensitivity and specificity. (Verma et al., 2015b; Chung et al., 2013; Jung et al., 2010; Torres-Chavolla and Alocilja, 2009; Lazcka et al., 2007) However, developing broad-spectrum biosensors using this strategy is cumbersome because each target requires the use of a unique biomolecule. An alternative method for developing versatile biosensors involves the use of a "chemical nose" where a set of interactions between the pathogen and sensors produces unique patterns of response, in a manner similar to the functioning of our sense of smell (Bunz and Rotello, 2010; Miranda et al., 2010; Rotello, 2009). Designing a "chemical nose" biosensor requires minimal prior knowledge of the analyte because the system can be 'trained' to recognize various analytes (Rotello, 2009). Such "chemical nose" sensors have been used for detecting various targets such as amino acids (Folmer-Andersen et al., 2006), proteins (De et al., 2009), carbohydrates (Wright et al., 2005), volatile organic compounds (Peng et al., 2009), bacteria (Li et al., 2014; Verma et al., 2014b; Wan et al., 2014; Phillips et al., 2008), and cancer cells (Rana et al., 2015; Bajaj et al., 2010; Bajaj et al., 2009; El-Boubbou et al., 2007).

Typically, nanoparticle-based "chemical nose" biosensors require the modification of nanoparticle surface with multiple ligands where each ligand is responsible for a unique interaction with the target (Wan et al., 2014; Bunz and Rotello, 2010). These interactions have only been studied in a limited manner (Abadeer et al., 2015; Yang et al., 2015; Hayden et al., 2012) and thus, their role in the performance of "chemical nose" biosensors is poorly understood. The use of multiple ligands limits the ability to study the nanoparticle-bacteria interactions because of increased complexity in synthesis. Here, we have utilized a single molecule, cetyltrimethylammonium bromide (CTAB)—a typical surfactant used for synthesis of gold nanoparticles—for providing electrostatic and hydrophobic interactions between nanoparticles with various

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morphologies and surface features of bacteria. CTAB-coated nanoparticles have previously been employed for detection of bacteria (Verma et al., 2014a, 2014b, 2015a), but the interactions between these nanoparticles and components of bacterial surface have not been studied.

Here, we demonstrate the crucial role of extracellular polymeric substances (EPS) in controlling the response of the "chemical nose" to the different bacterial species using lipid blot assays and transmission electron microscopy (TEM). Simulations of gold nanoparticle aggregation highlight that different types of aggregates are responsible for producing unique colorimetric responses to different species of bacteria. In the current study, we use this "chemical nose" to not only detect and identify eight different species of Gram-positive and Gram-negative bacteria at three different concentrations, but also discriminate between polymicrobial mixtures.

2. Materials and methods

2.1. Materials

Gold (III) chloride hydrate (HAuCl4 · xH2O), cetyltrimethylammonium bromide (CTAB), sodium borohydride, silver nitrate, hydrochloric acid, nitric acid, sodium hydroxide, L-ascorbic acid, Amersham™ Protran® Supported nitrocellulose (NC, 0.2 μm pore size) membrane, lipopolysaccharides (LPS-S) from *Pseudomonas aeruginosa* 10, rough strain (Rd) lipopolysaccharides (LPS-R) from *Escherichia coli* F583, peptidoglycan (PepG) from *Staphylococcus aureus*, and lipoteichoic acid (LTA) from *S. aureus* were purchased from Sigma-Aldrich (Oakville, ON, Canada). Trisodium citrate dihydrate was purchased from Thermo Fisher Scientific (Burlington, ON, Canada). Transparent, sterile 96-well microplates, scintillation vials (20 mL), BD trypticase soy agar (TSA) culture plates, BD TSA with 5% Sheep Blood (TSA II) culture plates, BD nutrient broth, sodium chloride (ACS grade), Nalgene sterilization filter units (0.2 μm pore size), calcium alginate swabs, and Amersham™ Hybond™ polyvinylidene difluoride (PVDF, 0.45 μm pore size) membrane were purchase from VWR (Mississauga, ON, Canada). 400 mesh formvar/carbon coated copper grids were purchased from Canemco Inc (Gore, QC, Canada). Cardiolipin (CL), L-α-phosphatidylglycerol (PG), and L-α-phosphatidylethanolamine (PE) from *E. coli* were purchased from Avanti Polar Lipids (Alabaster, AL, USA). *P. aeruginosa* (ATCC 9027), *S. aureus* (ATCC 6538), *E. coli* (ATCC 10798), *Achromobacter xylosoxidans* (ATCC 27061), *Delftia acidovorans* (ATCC 15668), *Stenotrophomonas maltophilia* (ATCC 13637), *Enterococcus faecalis* (ATCC 29212) and *Streptococcus pneumoniae* (ATCC 6305) were purchased from Cedarlane Labs (Burlington, ON, Canada). All procured chemicals were used without further purification. The 20 mL vials used for gold nanoseed synthesis were cleaned using 12 M sodium hydroxide and larger glassware was cleaned using aqua regia as described in published protocol (Liu and Lu, 2006).

2.2. Synthesis of gold nanoparticles

The gold nanoseed precursor was synthesized using a previously described simple two-step one pot process (Verma et al., 2014a, 2014b; Lu et al., 2010). Briefly, 60 μL of 0.1 M freshly prepared ice-cold sodium borohydride was added to 20 mL of a gold (III) chloride hydrate (2.4×10^{-4} M) and trisodium citrate dihydrate (10^{-4} M) solution under vigorous stirring. The sample was incubated overnight in the dark in ambient conditions, filtered (0.2 μm) and stored at 4 °C until use. To synthesize branched gold nanoparticles, a previously published procedure employing CTAB as a negative template was used with changes in the amount of silver nitrate to get a greater distinction between the morphologies of nanoparticles (Verma et al., 2014a, 2014b). Briefly, 210 mL of 7.33 mM CTAB and 1.46 mM CTAB were used for branched and spherical nanoparticles respectively. Gold (III) chloride hydrate (8.97 mL, 11 mM) was added to each CTAB solution, followed by silver nitrate (1.34 mL for branched nanoparticles and 0.67 mL for spherical nanoparticles, 10 mM) under moderate stirring. Then, L-ascorbic acid (1.44 mL, 100 mM) was added dropwise and the solution turned clear. The appropriate volume of gold nanoseed (2.24 mL for branched nanoparticles and 5.60 mL for spherical nanoparticles) was immediately added. The nanoparticles were purified by centrifugation at 10,000 rpm for 15 min resuspended in 1 mM CTAB solution. These two gold nanoparticle solutions were mixed (1:1 by volume) to obtain the purple "chemical nose" solution.

2.3. Bacterial culture

P. aeruginosa, S. aureus, E. coli, A. xylosoxidans, D. acidovorans, and *S. maltophilia* were inoculated on Trypticase Soy Agar (TSA) plates and incubated at 37 °C for 24 h. *E. faecalis* and *S. pneumoniae* were inoculated on TSA II plates and incubated at 37 °C for 24 h, where *S. pneumoniae* was placed in a 5% CO_2 environment. Bacterial cells were harvested using alginate swabs and suspended in 5 mL of sterile saline (2.55%) with nutrient broth (~0.006%) in a 15 mL centrifuge tube. In the case of *S. pneumoniae,* cultures from two TSA II plates were combined due to low OD_{660} values of the culture, which is used for normalization. Each bacterial strain was then washed seven times with 2.55% saline (with ~0.006% nutrient broth) by centrifugation at 4000 rpm for 10 min. The bacteria were then diluted to obtain an optical density at 660 nm (OD_{660}) of 0.10 ± 0.005 (~10^8 CFU/mL) (Dantam et al., 2011). The wavelength of 660 nm was chosen because it has previously been used for similar bacteria (Dantam et al., 2011). When the bacteria are added to gold nanoparticles, the solution is diluted 1:3 to obtain final $OD_{660} = 0.03$ for bacteria. The actual concentrations of bacteria were determined by plate count method and are summarized in Table S1. Other concentrations of bacteria were obtained by diluting the solutions 1:5 or 1:25 in 2.55% saline (with ~0.006% nutrient broth). In order to study the concentration dependent response (Fig. S7), the bacterial solutions were normalized to an OD_{660} of 1.0 ± 0.05 and then diluted 1:16, 1:32, 1:64, 1:128, 1:256 and 1:512. In the current study, all bacteria were grown at the same time and the effect of different growth phases on the colorimetric response was not studied. It is expected that the growth phases might alter the cell surface and hence, this aspect will be explored in future studies.

2.4. Removal of extracellular polymeric substances (EPS)

An EPS extraction protocol was used on *S. aureus, E. coli,* and *A. xylosoxidans* with a slight modification of published method (Liu and Fang, 2002). The bacteria were first incubated on TSA plates at 37 °C for 24 h. Bacterial cells were harvested using alginate swabs and suspended in 10 mL of sterile saline (2.55%) with nutrient broth (~0.006%) in 15 mL centrifuge tubes. 60 μL of formaldehyde was added to a 5 mL aliquot of the bacterial suspension and the rest of suspension was used as a control. The tubes were incubated at 4 °C for 1 h. Then, 4 mL of 1 M sodium hydroxide was added to the treatment tube and saline was added to control tubes and incubated at 4 °C for an additional 3 h. In order to remove EPS from the cells, the bacteria were washed by centrifugation at 4000 rpm for 10 min seven times.

The bacteria concentration was then normalized to obtain $OD_{660} = 0.10 \pm 0.005$ (~10^8 CFU/mL) (Dantam et al., 2011) and 100 μL of bacteria were added to 200 μL of purple "chemical nose"

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