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Towards point-of-care detection of polymicrobial infections: Rapid colorimetric response using a portable spectrophotometer

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ABSTRACT

Infectious diseases spread rapidly because current diagnostic methods are slow, expensive, and require technical expertise. Biosensors have recently been used as devices that can be deployed at the point-of-care for rapid and accurate diagnosis. Here, we show that a "chemical nose" biosensor based on gold nanoparticles can be coupled with a portable spectrophotometer to detect monomicrobial and polymicrobial solutions of pathogenic bacteria within 2 min of data collection. The design presented here exploits the rapid kinetics of gold nanoparticle aggregation around bacteria, which leads to a dramatic color change. The "chemical nose" produces unique signals based on the surface characteristics of the bacteria—such as the presence of extracellular polymeric substances, distribution of charged lipids, and localization of proteins—and hence provides a versatile platform for detection. We present a biosensor design that can easily be translated to the point-of-care because of its rapid response and simple output.

1. Introduction

Rapid detection of bacteria is crucial in curbing the spread of infectious diseases and preventing epidemics [1,2]. Current methods for detection of bacteria require considerable sample processing, because they detect either nucleic acids or proteins, which need to be extracted from the bacteria [3–5]. Culture-based methods are sensitive but slow because the growth of bacteria can require 1–5 days [2]. Additionally, most methods require sophisticated instruments and/or extensive technical training [1,2]. Rapid diagnosis of infectious diseases needs to be executed at the point-of-care with limited resources. Colorimetric responses are preferred in biosensors because they can be easily deciphered at the point-of-care [2,6,7]. Recently, portable scanners and smartphones have been used for measuring, analyzing, and reporting colorimetric responses when sensing analytes such as proteins [8,9], viruses [10], and bacteria [11].

Gold nanoparticles are playing an increasingly important role in providing a colorimetric response because their color depends on their aggregation state and their local environment [5,12]. Using gold nanoparticles for detecting pathogens typically requires biomodification with antibodies or aptamers for targeting specific analytes [2,5,6,13]. This "lock-and-key" approach is limited [14] because detecting multiple pathogens in a mixture requires a unique targeting biomolecule for each pathogen. A "chemical nose" approach provides a viable alternative to the conventional methods because the "chemical nose" can be trained for various analytes, including mixtures [14–16]. We have previously demonstrated that a "chemical nose" based on gold nanoparticles [17] can be used for identification of various unique pathogens once the system has been trained [18,19]. The gold nanoparticles aggregate in a unique manner around different species of bacteria due to the interactions of nanoparticles with bacterial extracellular polymeric substances [19], lipopolysaccharides (for Gram-negative species) [20], teichoic acids (for Gram-positive species) [21], and lipid domains formed around proteins [22] or in the presence of cationic molecules [23]. In order to implement this "chemical nose" at the point-of-care, here we have exploited the kinetics of the color change of gold nanoparticles in the presence of bacteria. The rapid color change provides sufficient data within 2 min to detect bacteria in monomicrobial and polymicrobial solutions. The portable spectrophotometer design used here has the potential to be translated easily to point-of-care use with the help of smartphone-based spectrophotometers [9,24].

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2. Materials and methods

2.1. Materials

Gold (III) chloride hydrate ($HAuCl_4 \cdot xH_2O$), cetyltrimethylammonium bromide (CTAB), sodium borohydride, silver nitrate, hydrochloric acid, nitric acid, sodium hydroxide, and L-ascorbic acid were purchased from Sigma-Aldrich (Oakville, ON, Canada). Trisodium citrate dihydrate was purchased from Thermo Fisher Scientific (Burlington, ON, Canada). Scintillation vials (20 mL), BD trypticase soy agar (TSA) culture plates, BD nutrient broth, sodium chloride (ACS grade), Nalgene sterilization filter units (0.2 μm pore size), and calcium alginate swabs were purchase from VWR (Mississauga, ON, Canada). 400 mesh formvar/carbon coated copper grids were obtained from Canemco Inc. (Gore, QC, Canada). *Pseudomonas aeruginosa* (ATCC 9027), *Staphylococcus aureus* (ATCC 6538), and *Escherichia coli* (ATCC 10798) 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 [25].

2.2. Spectrophotometer design

A standard optical extinction arrangement was used in the spectrophotometer design as previously described [26]. Briefly, a tungsten-filament lamp with fiber coupling (Ocean Optics HL-2000, Dunedin, FL, USA) was used as a light source and the light was collimated before passing through the cuvette containing nanoparticle solutions. The exiting light was collected into another fiber and directed to the portable spectrometer (Ocean Optics USB4000, Dunedin, FL, USA). Micro-volume disposable polystyrene cuvettes were used for the samples. The entire experimental setup was enclosed in a container to minimize external light and dust.

2.3. Synthesis of gold nanoparticles "chemical nose"

Gold nanoseeds were first synthesized using a published procedure [17–19,27,28]. 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. Branched and spherical gold nanoparticles were synthesized by modifying published procedure, where CTAB is used as a negative template [17,18]. The amount of silver nitrate used was increased to get a greater distinction between the morphologies of nanoparticles. 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 (14,087*g*) 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.

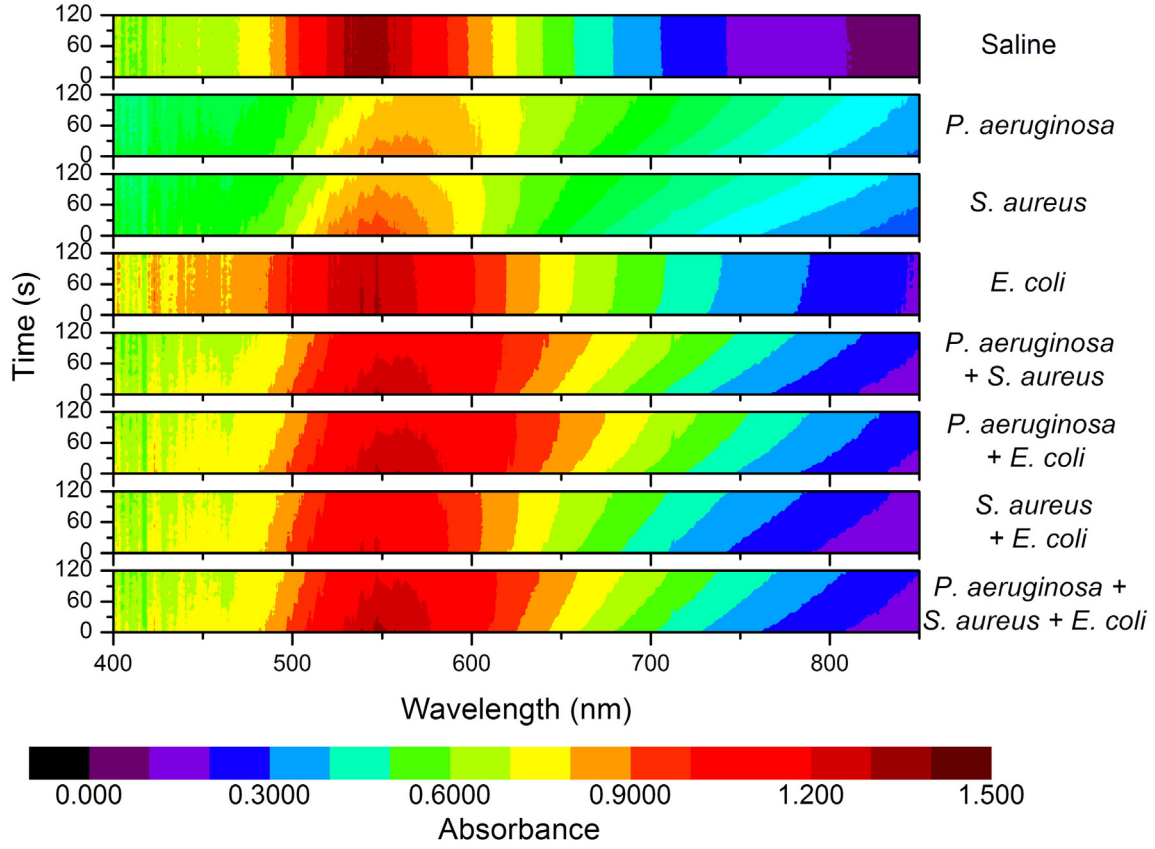

Fig. 1. Changes in absorption spectra of gold nanoparticles over time in the presence of bacteria: saline was used as a control, monomicrobial species were prepared such that the final OD_{660} of bacteria = 0.03 (approximately 5×10^7 CFU/mL), polymicrobial solutions were prepared by mixing 1:1 (v/v) or 1:1:1 (v/v/v) of the monomicrobial solutions. Initial time of zero indicates 1 min after addition of the nanoparticles. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)